

SEEDLINGS
THE RELATIONSHIP OF CARBOHYDRATE RESERVES TO
THE QUALITY OF BARE-ROOT *Pinus elliotii*
var. *elliotii* (Engelm.) SEEDLINGS PRODUCED
IN A NORTHERN FLORIDA NURSERY

By

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Three experiments explored relationships between carbohydrate reserves and quality of slash pine (Pinus elliotii var. elliotii) seedlings in a northern Florida nursery.

In the first experiment, growth of roots and shoots varied independently by season. Shoot dry weight increased most rapidly in summer, root weight in winter. Total weight increased from 2.95 g on November 2 to 3.95 g on February 24. Carbohydrate concentration averaged 6% from May to October, then increased steadily to 13.8% in late February. Root starch concentration increased from 1.1% in October to 11.8% in February. Physiological activity during the winter lifting season was therefore substantial.

In a second experiment, seedlings were undercut at four frequencies during November and December, then outplanted and periodically sampled. Outplanted seedlings suffered no loss in dry weight and reinitiated normal carbohydrate accumulation within 2 to 4 weeks. A temporary increase in sugar concentration occurred simultaneously with decreased starch. Root growth after outplanting was not dependent upon carbohydrate levels. Compared to the controls, undercutting resulted in 115% more new root mass, 68% more root starch during the first 12 weeks after outplanting, 24% higher survival, and 6% more first year height increment. Survival was linearly related to new root mass after outplanting ($R^2 = 0.48$).

In the third experiment, seedlings were subjected to five levels of water stress during December and January and were then outplanted. Pre-dawn xylem water potential of severely stressed seedlings averaged -1.3 MPa at lifting. Seedling morphology was not measurably affected. Sugar concentrations increased curvilinearly ($R^2 = 0.92$) with stress intensity, whereas starch decreased linearly ($R^2 = 0.83$) suggesting osmotic adjustment. The starch-sugar conversion was rapidly reversed by watering.

Nursery water stressing did not affect field survival but decreased first year height increment from 5.4 to 0.9 cm, probably related to impaired bud development. Height increment, however, was also correlated with total seedling starch ($R^2 = 0.67$).

GENERAL INTRODUCTION

Pine plantations provide an increasing portion of the supply base for the numerous wood products industries in the southeastern United States. Plantations are especially important to the pulp and paper industry, which is committed to short-rotation silviculture for fiber production. An essential part of the plantation system is the regeneration of harvested stands, including site preparation, planting, and cultural practices, such as fertilization and weed control. The success of this expensive enterprise depends upon the survival and early growth of planted seedlings.

The vast majority of seedlings planted in the Southeast are pines, which are usually produced in large-scale nursery operations. They are lifted bare-root and outplanted during the months of November to March. Slash pine (Pinus elliotii Engelm.) and loblolly pine (Pinus taeda L.) are by far the most common species. In fact, the combined nursery production of slash and loblolly pine exceeds the total production of all other species in the entire U.S. (Abbott and Fitch 1977).

Slash and loblolly pines are native to the Southeast and have a long history of successful plantation establishment.

Recently, however, there has been a growing concern about survival after field planting. Weaver et al. (1980) reported that overall average survival in pine plantations decreased from 82% in the early 1960s to 73% in the 1970s. Even though some landowners have not experienced this drop, prudence dictates concern for adequate survival.

Field survival depends upon many factors, notably weather, site conditions, and handling. Another crucial factor is use of a quality seedling, one which can cope with weather, site, and handling conditions so that initial survival and growth are maximized. If a nurseryman is to produce a quality seedling, however, he must be able to define what "quality" is. There must be specific seedling parameters which indicate whether a seedling is optimally plantable.

The definition of a quality seedling has proven elusive. For the past 50 years, nurserymen have used morphological characteristics as a measure of seedling quality. Such characteristics are height, root collar diameter, presence of secondary needles, presence of a dormant (winter) bud, woodiness of the stem, and the root/shoot ratio. Specific combinations of these characteristics have been used to define morphological grades (Wakeley 1954). Many studies indicate a strong relationship between morphological grades and seedling quality (Wakeley 1954, Switzer and Nelson 1967, Burns and Brendemuehl 1971, Blair and Cech 1974).

Yet, morphological grades are not always accurate indicators of seedling performance. At an early date Wakeley (1949) noted important exceptions to the morphological grade-quality relationship. He proposed that there were "physiological grades" of southern pine nursery stock which were the true determinants of quality and that these might or might not coincide with morphological grades. The inadequacy of morphological grades in determining seedling quality has been further indicated by Stone (1955), Shoulders (1960), Stone et al. (1962), Stone and Jenkinson (1971), Blair and Cech (1974), and Chavassee (1977). It is now generally accepted that seedling quality is influenced by physiological condition (Sutton 1979, Brissette et al. 1981).

Research on the numerous physiological factors that may be related to seedling quality has concentrated on osmotic potential, nutritional balance, endogenous plant growth regulators, and carbohydrate reserves. A hypothesized relationship between carbohydrate reserves and slash pine seedling quality holds that root regeneration potential of outplanted seedlings is dependent upon carbohydrate reserves (Wakeley 1949). Recent research has lent some support to this hypothesis (Barnard et al. 1981). The lack of a strong photoperiod influence or chilling requirement for the spring shoot flush of slash pine seedlings (Wakeley 1954, Kaufmann 1977, Fisher 1981), as opposed to many other pine species, strengthens this hypothesis inasmuch as it suggests reduced hormonal involvement. Even so, there has been little work on the

contribution of carbohydrate reserves to seedling quality and several basic issues have yet to be resolved.

The overall objective of the present study was to investigate the relationship between carbohydrate reserves and seedling quality for nursery-grown slash pine (Pinus elliotii var. elliotii Engelm.) in northern Florida. The study concentrated on four issues.

- A. To determine the "normal carbohydrate levels in nursery-grown slash pine seedlings
- B. To determine the influence of some cultural treatments on "normal" carbohydrate levels
- C. To determine what happens to these reserves after outplanting
- D. To relate carbohydrate reserves to survival in the field

During the course of this study, a freezer malfunction resulted in the thawing of certain samples and their subsequent loss for the purpose of carbohydrate analysis. While this reduced the total amount of information available, enough sound data were produced to address the specified objectives.

LITERATURE REVIEW

Introduction

The ability of nursery-grown seedlings to survive outplanting depends on the rate of new root production. As Switzer and Nelson (1967, p.5) pointed out, "the typical planter reasons that trees must survive to grow, while in reality they must grow to survive."

Several studies relate seedling field performance to Root Growth Capacity (RGC). Both survival and height increment of boreal conifer seedlings have been related to RGC (Sutton 1980). Burdett (1979) showed that in certain circumstances, survival of lodgepole pine (Pinus contorta Dougl.) is largely dependent on its RGC. Rapid root growth immediately after outplanting is also critical to the survival of southern pines (Woods 1959, Switzer and Nelson 1967, Kozlowski 1979). Wakeley (1954, p.123) states that "high initial survival seems to depend, perhaps even more than that of pines planted in other regions, upon formation of considerable new root tissue after planting."

Lateral Root Formation

The physiology of lateral root formation is complex and incompletely understood. Wightman and Thimann (1980) showed that for Phaseolus the increase in lateral root growth after the root tips are removed is a result of a specific promotor (an auxin), which is produced in the leaves (cotyledons) and moves acropetally to concentrate in the most apical segment of the decapitated roots. Growth of lateral root primordia is inhibited by substances produced in the root tip. Removal of this inhibitory effect by root decapitation did not increase the number of root primordia but allowed growth of existing primordia. Lateral root extension, therefore, resulted from a synergistic effect of a promoter produced in the cotyledons and an inhibitor produced in the root tips.

The effect of IAA on lateral root emergence is directly connected to carbohydrate physiology. Altman and Wareing (1975, p.37), also working with Phaseolus, found that IAA affected carbohydrate transport directly, as well as inducing the formation of a stronger "sink" in the root primordia. These two effects were independent. One of the proposed effects of leaf-produced IAA was a stimulation of the conversion of starch to sugar. The authors stated that "where root initiation is easy, carbohydrates transported from the leaves are the main limiting factor, rather than unknown 'co-factors' of hormonal nature."

One of the possible mechanisms whereby carbohydrates activate primordia is through an effect on the mitotic cycle. With Vicia faba, Van't Hof et al. (1973) showed that carbohydrate starvation caused cell arrest in the G1 and G2 phases of mitosis. The longer the starvation, the more difficult to re-initiate mitosis. Also, the first metabolic activity after sucrose feeding of starved cells was protein synthesis.

One of the first events during root primordia development is increased activity of enzymes directly involved in carbohydrate physiology (Haissig 1982b). Application of naphthaleneacetic acid (NAA) to Pinus banksiana (Lamb) seedling cuttings caused an increase in the activity of glyceraldehyde 3-phosphate dehydrogenase (Haissig 1982a). This increase was temporally associated with callus formation. Moreover, development of the callus primordium was positively correlated with total carbohydrate. Sugars produced in the needles were translocated and concentrated in the stem, with the exogenously applied auxin directing the basipetal transport of these sugars.

Stoltz (1968) found that rooting ability of two difficult-to-root cultivars of Chrysanthemum was correlated with total carbohydrate concentrations. The cultivar with the highest carbohydrate reserves produced more roots regardless of preparation date.

Root cuttings of apple cultivars also showed maximum rooting ability when carbohydrate contents were greatest (Robinson and Schwabe 1977). Carbohydrate contents of apple tree roots vary according to season. Levels of both carbohydrate and rooting ability were highest in autumn and winter. During the growth of these roots, polysaccharide reserve concentrations declined by 30 and 40%, presumably being used for root and shoot formation. The authors held that a "minimum polysaccharide threshold level" of approximately 30% of root dry weight was necessary for survival. The same study found evidence of carbohydrate X IAA interactions, however, and the authors felt that a suitable balance of each was necessary for optimum rooting potential.

Similarly, a hormone/carbohydrate balance was also necessary for root formation on etiolated stem segments of Populus (Nanda et al. 1971, p.391). The effectiveness of an auxin in promoting stem segment rooting depended upon the carbohydrate produced in leaves. The authors concluded that the "rooting of 2.5 cm long segments of Populus nigra is limited primarily by nutritional factors."

Seasonality of Carbohydrate Physiology

Woody species, as a rule, have seasonal fluctuations in carbohydrate concentrations, especially starch. The amount of photoassimilates going into storage increases in the fall

(Kruegar and Trappe 1967, Ursino et al. 1968, Schier 1970, Nelson and Dickson 1981). Kruegar and Trappe (1967) found that both sugar and starch in Douglas-fir (Pseudotsuga menziesii Franco) seedlings fluctuate considerably by season, with winter concentrations of sugars three times those in summer. The same was true for starch except that concentrations peaked in early spring.

Winter carbohydrate reserves may serve to fuel the flush of growth in the spring, especially in deciduous species (Kramer and Kozlowski 1979). Utilization of carbohydrate reserves for the sprouting of aspen sucker shoots has been documented (Schier 1981, Fitzgerald and Hoddinott 1983). Decreases in total carbohydrate concentrations of seedling shoots during the spring shoot flush have been demonstrated in Douglas-fir (Kruegar and Trappe 1967). On the other hand, a study with 5 year old balsam fir (Abies balsamea L.) seedlings transplanted into a nursery soil found the spring flush independent of reserves (Little 1974). In this case, current photoassimilates supported the spring top growth.

Apparently, environmental conditions influence the balance between use of current photosynthate and reserves during the spring flush. In the case of young red pines (Pinus resinosa Ait.), unfavorable temperatures and moisture may delay the re-establishment of the photosynthetic capacity of old needles and

thus increase the importance of reserves for the spring flush (Gordon and Larson 1970).

Although Kruegar and Trappe (1967) found shoot reserves used for new shoot expansion, there was no parallel decrease in root starch concentrations during spring root growth. The authors concluded that this indicated a "steady-state" situation, with sugars from the top supporting root extension. Schier (1970), however, showed that red pine root expansion from January to July must have been at the expense of root reserves. Ursino et al. (1968) also discovered little evidence for spring translocation of photoassimilates to the roots in white pine (Pinus strobus L.).

A number of studies have demonstrated seasonality of RGC in forest nursery seedlings. Stone et al. (1962, p.296) found that Douglas-fir nursery seedlings "displayed marked periodicity in their root--regenerating potential," with winter-lifted seedlings having much higher RGC than summer-lifted seedlings. Lavender (1964) felt that early lifting decreased RGC due to disruption of the physiological process of dormancy. Stone and Jenkinson (1971) reported that the ability of ponderosa pine (Pinus ponderosa Laws) seedlings to regenerate roots is dependent upon the number of hours that seedlings are exposed to low air temperatures in the nursery. A minimum of 1500 hours below 10°C is needed to insure a high root growth capacity. Spring RGC for northern hardwoods is also controlled by a chilling factor

(Farmer 1978). Exogenous applications of IAA or gibberellins did not improve the RGC of either Douglas-fir (Lavender and Hermann 1970) or ponderosa pine (Zaerr 1967).

The relationship between dormancy controls, such as chilling, and carbohydrate levels is quite complex. How they interact to influence RGC of seedlings has not been completely elucidated. Most of the studies on this topic have concentrated on species with strong chilling requirements.

Ronco (1973, p.213), for example, stored 3-0 Engelmann spruce (Picea engelmannii Parry) seedlings 4 months at 1-2°C. Total carbohydrates decreased from 265 to 120 mg/g dry weight, and upon outplanting after storage survival was very poor. A second group of seedlings that were lifted from holding beds and planted immediately (no storage) had total carbohydrate reserves of over 300 mg/g dry weight and survived better. When comparing the performance of both groups, Ronco concluded that "survival may be adversely affected by carbohydrate concentrations below certain threshold levels when trees are planted."

McCracken (1979) stored Pinus mugo Turra and Pinus radiata D. Don for up to 18 weeks at 1°C. At 6, 12, and 18 weeks, seedlings were sampled for laboratory analysis as well as potted for growth analysis. Total carbohydrates decreased "markedly" for both species, especially Pinus radiata during cold storage. Furthermore, in both species, decreases in carbohydrate levels were

associated with increases of new tissue after potting, indicating that reserves were used during the growth process. This evidence, along with a previous finding that cold storage reduces photosynthetic efficiency (McCracken 1973), caused the author to conclude that stored carbohydrates form a vital resource for growth after cold storage and transplanting.

Ritchie (1982) obtained somewhat different results with Douglas-fir. Seedlings were lifted periodically from November to March, stored at 2°C for different lengths of time, and their RGC and carbohydrate content were determined. Total nonstructural carbohydrate (TNC) decreased during storage, but RGC did not always do so. For example, with January-lifted seedlings, TNC decreased during the first 6 months of storage, whereas RGC (of planted seedlings) actually increased. The author concluded that changes in RGP are not driven by changes in carbohydrate concentrations.

Van den Driessche (1979) presented similar results for red pine and white spruce (Picea glauca Voss) seedlings. Root growth capacity was low in fall and spring but high in midwinter. These differences in RGC did not appear to be related to carbohydrate reserves. The results also indicated that current photosynthate was essential for new growth in Pinus resinosa.

Recent work with hardwoods has indicated a seasonal fluctuation of starch reserves (Rose and McGregor 1982, Rietveld et al. 1982). With four hardwood species in southern Illinois, Rietveld et al. (1982) found a weak, but consistent, association between later lifting dates, which had higher root starch concentrations and greater survival and/or growth in the field. Unfortunately, TNC was confounded with lifting date, which did not allow for the separation of their individual effects upon field performance.

Stress Physiology

The process of lifting, storage, transport, and planting imposes various types of stress on nursery-produced seedlings. The chief stress factor is desiccation, which can occur at any time before or soon after planting. It is evident that the physiology of outplanting is to a great extent the physiology of water-stressed seedlings.

Increasing levels of water stress usually result in decreasing photosynthetic capacity. A water potential of -0.4 MPa has been shown to reduce the photosynthetic capacity of loblolly pine, with assimilation becoming negligible at -1.1 MPa (Brix 1962). This effect was caused by closure of the stomates, which reduces transpiration as well as photosynthesis. Upon rewatering, tree seedlings re-initiate photosynthesis. The period of

time required by the plant to return to 100% efficiency, however, depends upon the length and intensity of the previous water deficit, species, and humidity (Brix 1962, Zavitkovski and Ferrell 1970, Kramer and Kozlowski 1979). Plants rewatered after water stress may begin transpiring before photosynthesizing, indicating a period when the photosynthetic mechanism is being repaired (Kramer and Kozlowski 1979).

The ability to tolerate water stress is an important component of the adaptability of a species. A species may be limited to moist sites by sensitivity of stomata to water stress and poor recovery of leaf gas exchange following rewatering (Davies and Kozlowski 1977). While slash pine is a wet-site species (Fisher 1981), it possesses the xerophytic adaptations of a heavy cuticle and sunken stomates characteristic of the genus. Moreover, it can be found growing naturally alongside longleaf pine (Pinus palustris L.) on drier sites.

Water stress has been shown to cause specific changes in the carbohydrate physiology of tree seedlings. Decreases in starch simultaneous to increases in sugars during drought stress have been reported for sugar maple (Acer saccharum Marsh.) (Parker 1970) and black oak (Quercus velutina Lam.) (Parker and Patton 1975). In the case of sugar maple, the total reserves were not affected, indicating a transfer of carbohydrate from the storage form (starch) to sugars. Similar changes occur in the inner bark of loblolly pine (Hodges and Lorio 1969) and the tops of cotton plants (Eaton and Ergle 1948).

The increase in sugar concentrations resulting from water stress may serve a number of purposes, of which two are of prime consideration. First, an increase in free sugars increases the levels of readily available substrate for respiration. Brix (1962) found that when loblolly pine seedlings were subjected to increasing levels of water stress, respiration first decreased, then dramatically increased. At one point (-2.8MPa plant water potential), respiration was 140% of the rate at soil field capacity.

A second effect of increased free sugars during water stress is to lower the osmotic potential of cell solution and therefore maintain cell turgidity as soil water becomes unavailable. Carbohydrates, organic acids, and inorganic ions can be used for osmotic regulation (Kramer 1983). That solute accumulation occurs in woody plants subjected to moisture stress was demonstrated by Osonubi and Davies (1978). The authors stressed young seedlings of English oak (Quercus robur L.) and silver birch (Betula verrucosa Ehrl.) and observed a more negative leaf solute potential as soil water content decreased. The efficiency of osmotic regulation varied between species. The oak was able to lower solute potentials and thus maintain turgor pressure and keep stomates open at lower soil water potentials. Unfortunately, the constituents of the solutes were not assessed in this study, and it is not known whether organic or inorganic ions were the principal influences upon changes in osmotic potential.

Root pruning (undercutting) is a part of the standard lifting process. This results in substantial loss of the root system, especially non-suberized root tips as well as damage to the entire root system when the soil is lifted as the blade passes underneath. Undercutting would therefore induce seedling moisture stress.

By successively root pruning over a period of time well before lifting, seedlings are rendered more drought tolerant. Root "wrenching" is a standard cultural technique in the production of radiata pine in New Zealand (Van Dorsser and Rook 1972), and Caribbean pine (Pinus caribaea Mor.) in Australia (Bacon and Hawkins 1979). These studies and others (Shoulders 1963, Tanaka et al. 1976) have found that the systematic stressing of seedlings by root pruning and/or wrenching in the nursery improves field performance.

The reason for the improved performance is not clear but may be related to morphological changes resulting from undercutting. Root pruning results in a more fibrous root system, smaller foliage volume, and a reduced height, which makes for a more favorable root/shoot ratio (Rook 1971). Physiological changes in radiata pine include increasing amounts of photosynthate sent to the pruned roots, which causes higher concentrations of reducing sugars and starch (Rook 1971).

Bacon and Bachelard (1978) found that root wrenching Caribbean pine changed not only seedling morphology but also the physiological response of seedlings to water stress after outplanting. Seedlings that had been intensively wrenched kept stomates open upon potting, whereas control seedlings did not. Control seedlings stopped transpiring within two days after potting even though soil moisture was adequate. On the other hand, while wrenched seedlings declined in transpiration rate after potting, they did not completely stop. Furthermore, wrenched seedlings showed the highest net photosynthetic rate during the 15-day measurement period after potting. The results indicate that the seedling's ability to photosynthesize after transplanting is related to the level of conditioning in the nursery.

Because seedlings are normally lifted in the winter, they may be exposed to low soil temperatures after outplanting. Nambiar et al. (1979, p.1119) found that low soil temperature slowed the initiation and elongation of new roots of radiata pine. The study indicated that the water stress which occurred during the first several weeks after planting was "due primarily to the suppressive effect of low soil temperature on root regeneration." The authors maintained the soil temperature of potted seedlings at 5, 10, or 20°C. The seedling stress levels varied among these soil temperatures even though the pots were watered twice weekly.

Undoubtedly, some water was absorbed through suberized roots (Kramer 1946, Chung and Kramer 1975), but the growth of new roots was necessary to establish an adequate internal water balance.

Root Regeneration in Southern Pines

Wakeley (1949) was one of the earliest investigators to relate carbohydrate levels to planting stock quality of southern pines. He found that either shading or defoliating longleaf (Pinus palustris Mill.) and slash pine seedlings in the nursery over a period of 3 to 12 weeks affected the survival after outplanting. These experiments were repeated over four successive years. The more extreme treatments reduced survival by 45 to 97% in each of the 4 years. Staining with potassium iodide indicated that both severely shaded and defoliated seedlings contained little or no starch.

Gilmore (1961) was able to demonstrate that shading reduced carbohydrate reserves of loblolly pine, and correlated survival of the shaded seedlings with their root carbohydrate reserves. In later publications (1964, 1965), he reported no correlation between carbohydrate reserves and survival or root growth after outplanting normal (unshaded) nursery-run seedlings. He concluded that a "growth promoting substance" produced in the shoots provide roots with a necessary stimulus for root growth.

Hay and Woods (1968, 1975, 1978) examined the interaction between root deformation and root regeneration in loblolly pine. When seedlings were planted in "deformed" positions, such as "J rooting," free sugars tended to concentrate at the point of curvature. Translocation was therefore impeded, with the root deformation acting as a phloem girdle. Lateral root development was most visible at the bottom of the "J" and in association with the increased carbohydrate concentrations.

The only recent work concerned with carbohydrate reserves in slash pine seedlings is that of Barnard et al. (1981). The authors compared root starch concentration with survival after out-planting for nursery-run seedlings from each of four nurseries in northern Florida. Low survival appeared to be associated with low root starch concentrations. The authors concluded that the relationship of low starch with low survival was consistent and warranted further investigation.

CHAPTER I
CHANGES IN MORPHOLOGY AND CARBOHYDRATE RESERVES
DURING THE NURSERY SEASON

Introduction

In order to relate the field performance of slash pine seedlings to their carbohydrate reserves, some basic information on seedling carbohydrate physiology is required. For example, what are "normal" levels of carbohydrate reserves for slash pine seedlings? Are these reserves found in amounts sufficient to contribute to survival after outplanting? Also, is there an appreciable seasonal variation in carbohydrate reserves? If so, this would suggest variability in the potential contribution of such reserves to field performance.

A study to follow morphological and total non-structural carbohydrate (TNC) development over time was established in an industrial forest nursery near Archer, Florida. The quantity of reserves, their changes over time, and their general relation to the morphological characteristics of the plant were followed.

Materials and Methods

Field Procedure

The nursery selected for this study has produced around 20 million bare-root loblolly and slash pine seedlings per year since beginning production in 1971. The area receives an annual average of 1370 mm rainfall, evenly distributed except for a late fall- early winter dry period, with November averaging less than 50 mm (Dohrenwend 1978). Mean January and July temperatures are 14.4 and 27.3°C, respectively. The soil is a moderately well drained Grossarenic Paleudult, which has been graded to improve drainage. A clay layer is found from 100 to 120 cm depth in the area of the study.

The sampling area was managed according to standard procedures of this nursery. The seed was from a single open-pollinated seed orchard of improved slash pine. After soil fumigation, seed were sown mechanically at a rate that would produce about 30 seedlings per square foot of bed space. Fertilization consisted of a plowdown application of 0-20-10 at 672 kg/ha, and subsequent applications of 10-5-5 liquid fertilizer three times during the summer. The liquid applications were made through the sprinkler system with an objective of 16.8 kg/ha of elemental nitrogen at each application. Bayleton® (triademeton) systemic fungicide was sprayed on May 5 and 20 and on June 10.

Seedlings were sampled throughout the entire growing season, beginning 4 weeks after sowing on April 28, 1982, and terminating on March 8, 1983. The experimental area consisted of two nursery beds 173.7 m (570') long by 1.2 m (4') wide, with eight seedling rows per bed. Eight 9.1 m (30') long replicate plots were evenly spaced along these two beds (Figure 1-1). Every 2 weeks, at least 20 seedlings were lifted from each replication. As there were eight replications and eight rows per bed, a different row was sampled from each replication at each time; thus the entire bed width was represented. Lifting was done with a shovel, attempting to get the entire root system.

Laboratory Procedure

After lifting, the seedlings were placed in plastic bags and covered with ice in a cooler. They were then brought to the laboratory and stored frozen (-20 to -10°C) until measured.

Upon removal from the freezer, the seedlings were immersed in water for thawing and washing (3-5 minutes). Several morphological characteristics were then measured on 20 seedlings from each replication. These characteristics included shoot length from root collar (height), diameter at root collar (DRC), length of the primary root from the root collar (measured only when the primary root was intact), and presence or absence of a dormant (non-active) bud in seedlings sampled after October. Ten of

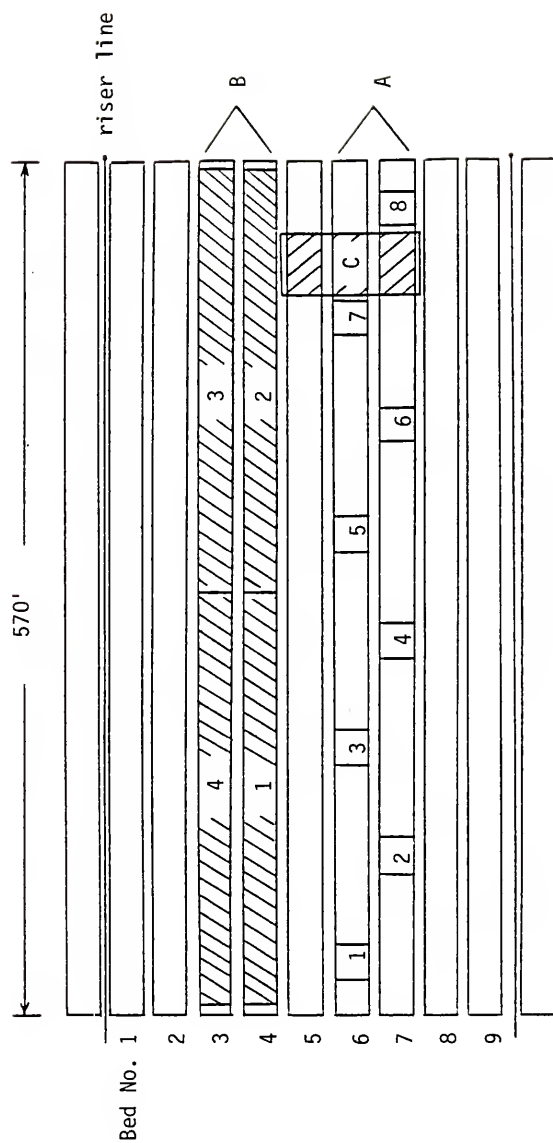


Figure 1-1. Section 14 of the Archer nursery indicating experiment locations (numbers indicate replications).

- A. Seasonal carbohydrate reserve study
- B. Root pruning study
- C. Water stress study

these seedlings were blotted dry and both green and oven dry (105°C) weight determined. The remaining ten were used for carbohydrate determination. Root and shoot were separated and dried at 105°C for 1 hour followed by 70°C for 23 hours. Thereafter, the tissue was milled to pass 40 mesh and stored in air-tight vials. A total of 336 carbohydrate analyses was required for analyses of roots and shoots of eight replications and 21 sample dates.

The analytical procedure followed that of Rowe (1981). Free sugars were extracted from a 100 mg sample with 80% ethanol. The sample was then subjected to enzymatic hydrolysis of the starch for 2 hours using amyloglucosidase (Sigma, from *Rhizopus*). Both the ethanol solution of free sugars and the enzyme-produced glucose solution were analyzed colorimetrically using anthrone reagent dissolved in 80% concentrated sulfuric acid. Their sum is the total non-structural carbohydrate (TNC) of the sample).

Statistical Analysis

Values of the eight replications on each sampling date were averaged and expressed on a per seedling basis. The averaged values were graphed to follow parameter changes over time. Also, average seedling values of root and shoot dry weight were used for allometric analysis, which plots the logarithm of shoot dry weight against the logarithm of root dry weight. The MEANS and

General Linear Model procedures of the Statistical Analysis System (SAS) were used for data analysis.

Because of a freezer malfunction, stored samples taken on November 2, December 1, and January 10 did not produce acceptable carbohydrate data. Morphological data from these samples were utilized, however.

Results

Morphological Characteristics

There were three distinct phases of height growth: an initial period of slow growth, rapid summer growth, then a decreasing growth rate in fall and winter (Figure 1-2). In the 6 weeks from July 8 to August 19, the seedlings grew 13.2 cm, or 44% of the total height accrued during the entire study. A second period of rapid height increase began in late February.

From first sampling on May 27 to July 8, a period when shoot growth was relatively slow, the primary root was rapidly elongating. By July 8, primary root length was 30.5 cm, or 86% of the total length at the termination of the study. Primary root elongation slowed during July and was generally associated with a decreased length in the zone of elongation and increased degree of suberization. This did not appear to be related to any specific soil changes.

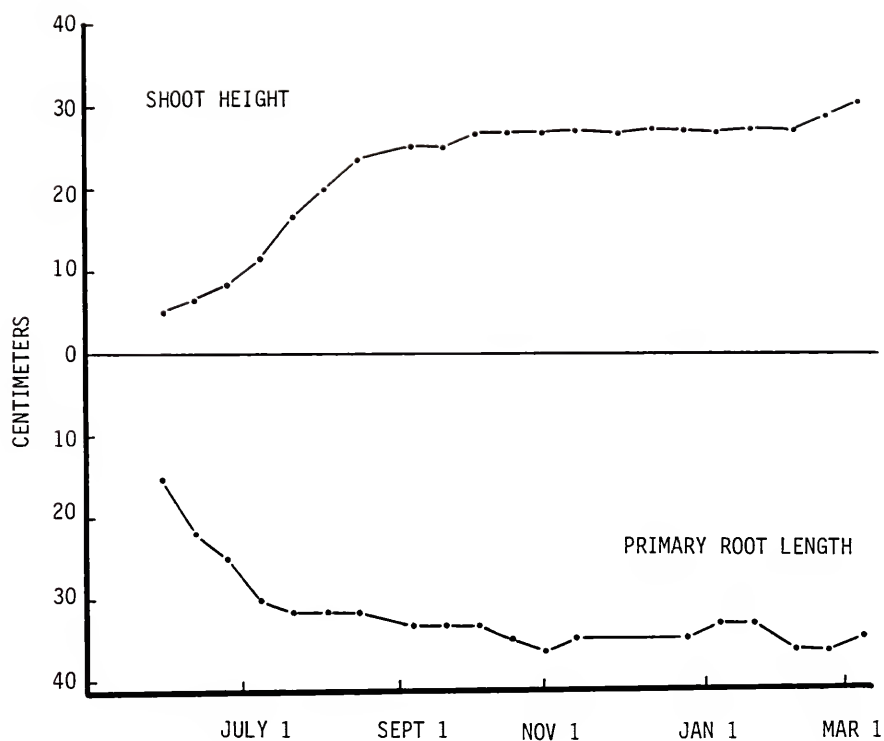


Figure 1-2. Average shoot height and primary root length. Each point represents 160 seedlings. Maximum $S_{\bar{x}}$ for any single point is .54 cm for height and 2.2 cm for primary root depth.

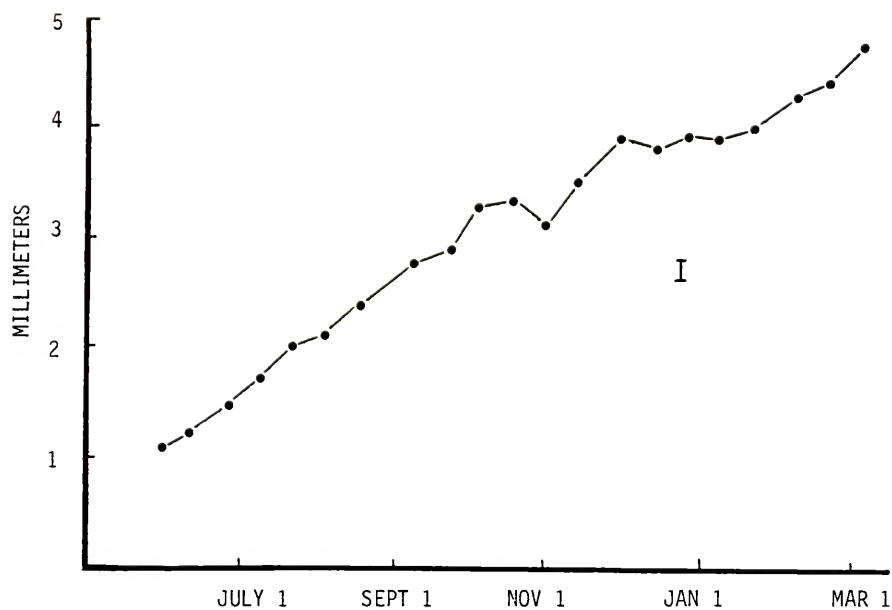


Figure 1-3. Average root collar diameter. Each point represents 160 seedlings. Bar represents the maximum $S_{\bar{x}}$ for any single point.

While top height and primary root length remained fairly stable after a certain period, seedling root collar diameter (DRC) continued to increase throughout the study period (Figure 1-3). Although the increase is approximately linear indicating that diameter growth occurred at a steady pace, there was a slight decrease during the winter.

Like DRC, seedling dry weight increased throughout the entire study period (Figure 1-4). Increases of root and shoot varied by season. There was an early development of root mass followed by the summer and fall shoot increase, which overlapped the subsequent period of fall and winter root growth. Mean shoot dry weight increased from 2.3 g on November 16 to 2.7 g on February 24, an increase of 18%, whereas root weight went from .62 g to 1.22 g, an increase of 97%. Changes in relative growth rate of the two organs caused a strong U-shape in the root/shoot ratio over time (Figure 1-5).

Allometric analysis is often used to analyze the relative growth rates of plant organs (Leopold and Kreidiman 1975). A regression of log shoot dry weight with log root dry weight has an R^2 of .94 (Figure 1-6). Despite the high correlation, the line is obviously not linear, especially at the upper end.

Carbohydrates

Free sugar concentrations of both roots and shoots were higher during the winter than in the summer by about 20 mg/g (2%)

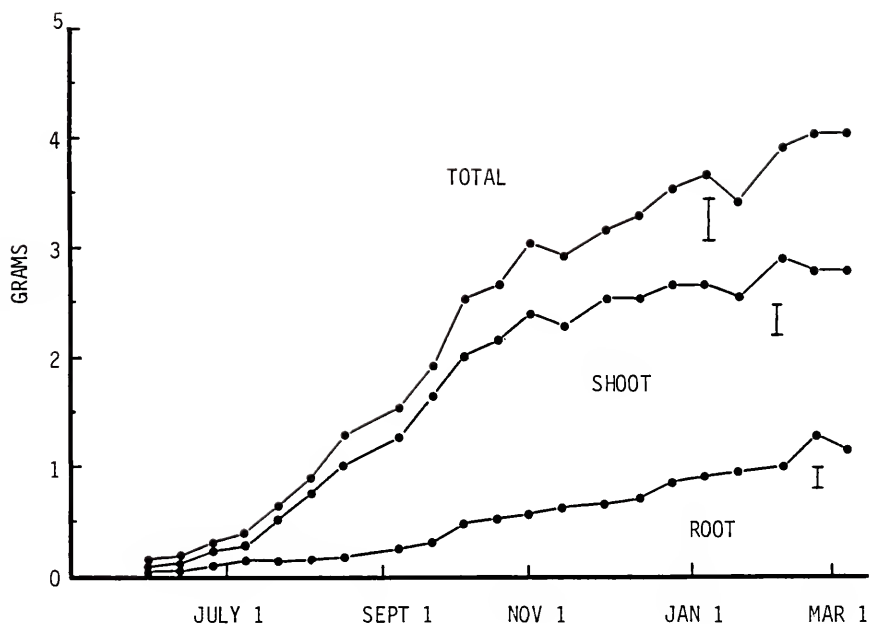


Figure 1-4. Average root, shoot, and total dry weight per seedling. Each point represents 160 seedlings. Bars are the maximum $S_{\bar{x}}$ for any single point.

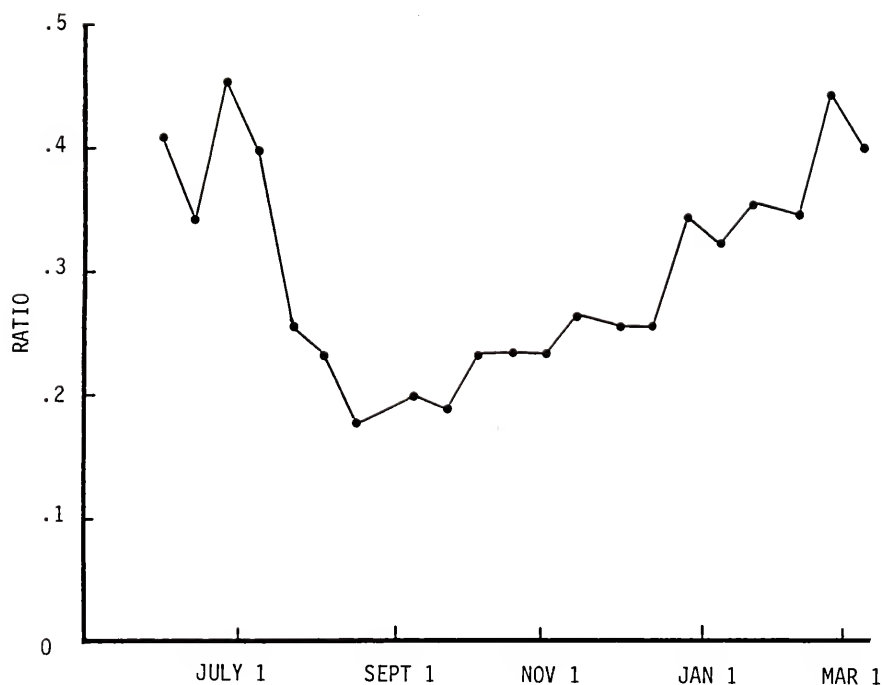


Figure 1-5. Average root/shoot ratio per seedling (dry weight basis). Each point represents 160 seedlings. Bar represents the maximum $S_{\bar{x}}$ for any single point.

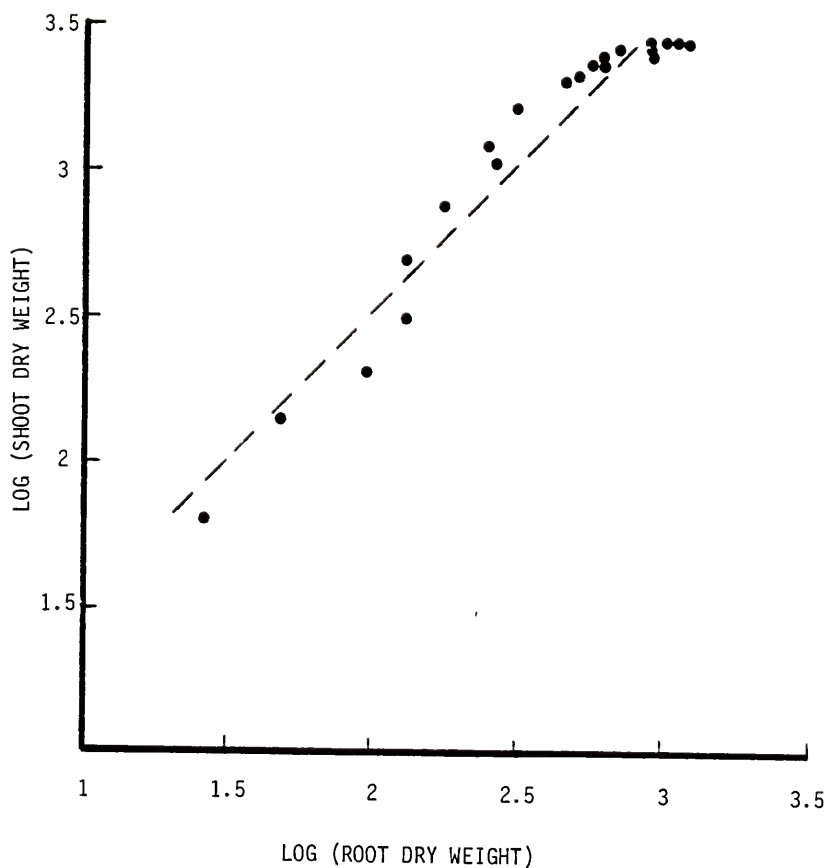


Figure 1-6. The relationship of log shoot dry weight to log root dry weight for biweekly samples. (The linear regression is $y = 0.51 + 1.01x$, $R^2 .94$.)

on a dry weight basis (Figure 1-7), with concentrations beginning to increase in October and peaking on January 24. In general, free sugars in the shoots and roots remained around 5% and 3%, respectively, during the summer and then increased to 7% and 5% during the winter.

The considerable fluctuation of sugar concentrations between sampling dates in early summer may be related to seedling size. For example, on June 11 the seedlings were only 6.5 cm tall and had a proportionately large photosynthetic area. The diurnal variation of photosynthesis would result in higher sugar concentrations if sampled in midafternoon as opposed to early morning (Kramer and Kozlowski 1979). As seedlings get larger, these daily concentration changes are buffered by the proportionally greater amount of woody tissue.

The late fall and winter increases in starch concentration were much larger (Figure 1-8). Root starch concentration increased from an average of 1% in late October to 12.8% on February 28, whereas shoot concentration rose, from around 1% to 4.3% in the same period.

The relative contribution of starch and sugar concentrations to seedling TNC becomes clearer when placed in the perspective of the whole plant (Figure 1-9). Sugar concentrations of the whole plant are higher than starch concentrations until late winter (February 10). Because sugars are concentrated in the shoot, which is the larger fraction of total seedling weight, their contribution to the total seedling is proportionately large.

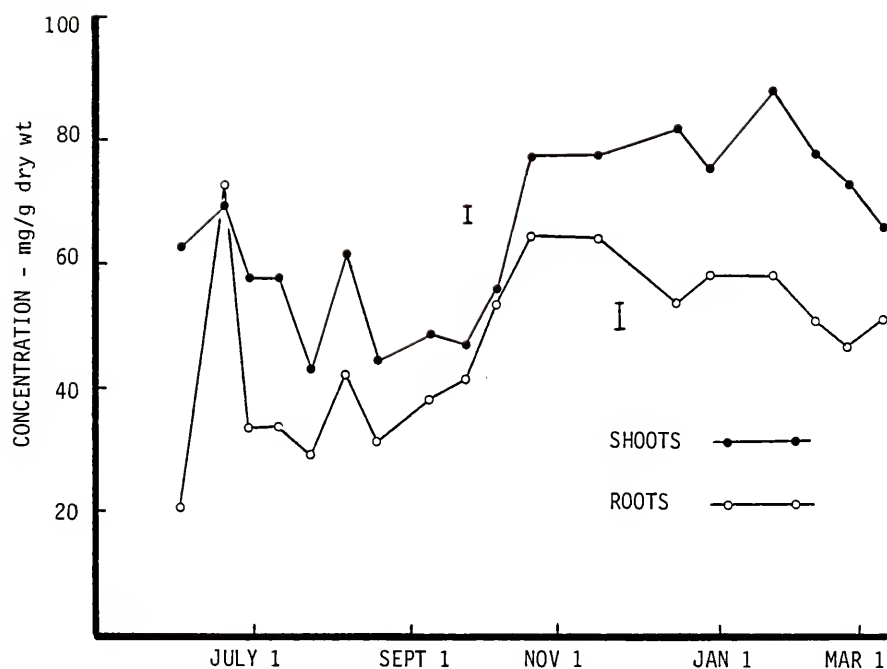


Figure 1-7. Average seedling sugar concentrations. Each point is an average of eight composite samples of 10 seedlings. Bars represent the maximum $S_{\bar{x}}$ for any single point.

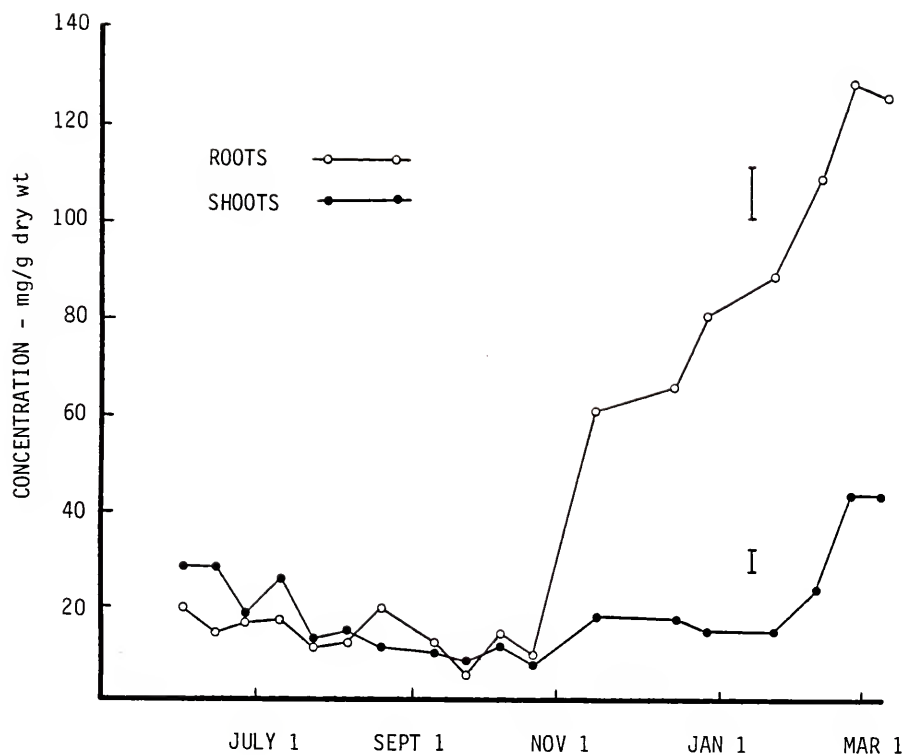


Figure 1-8. Root and shoot starch concentrations. Each point is an average of eight composite samples of 10 seedlings. Bars represent the maximum S_x for any single point.

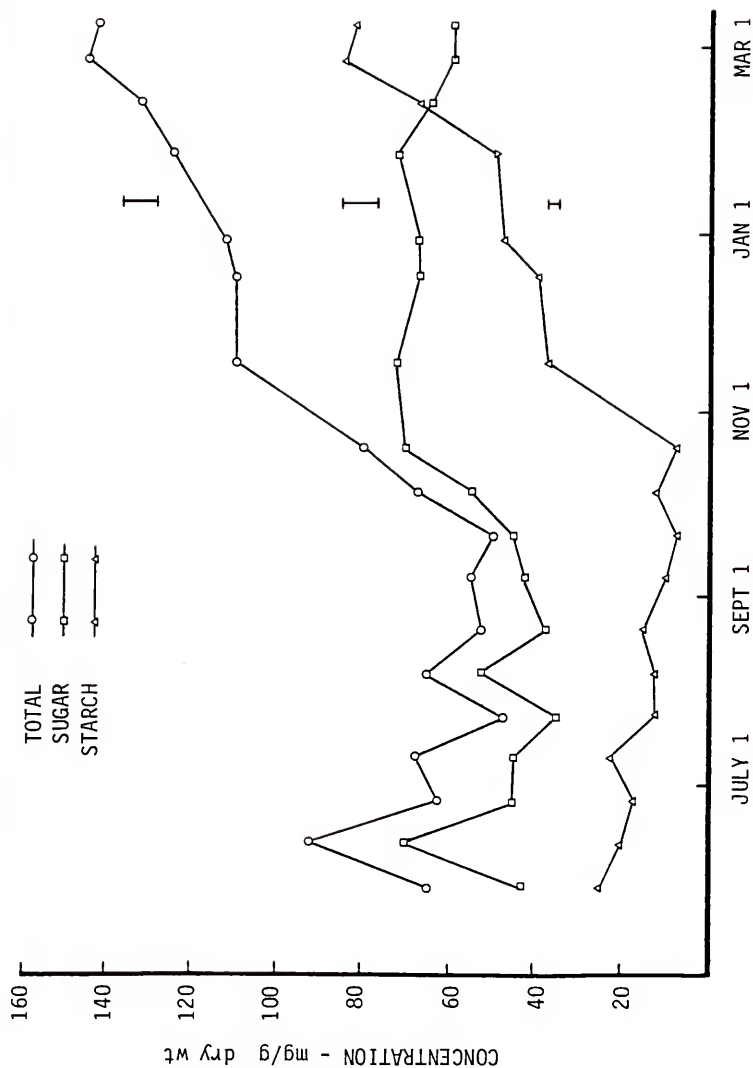


Figure 1-9. Sugar and starch concentrations of entire seedlings. Each point is an average of eight composite samples of 10 seedlings. Bars represent the maximum $S_{\bar{x}}$ for any single point.

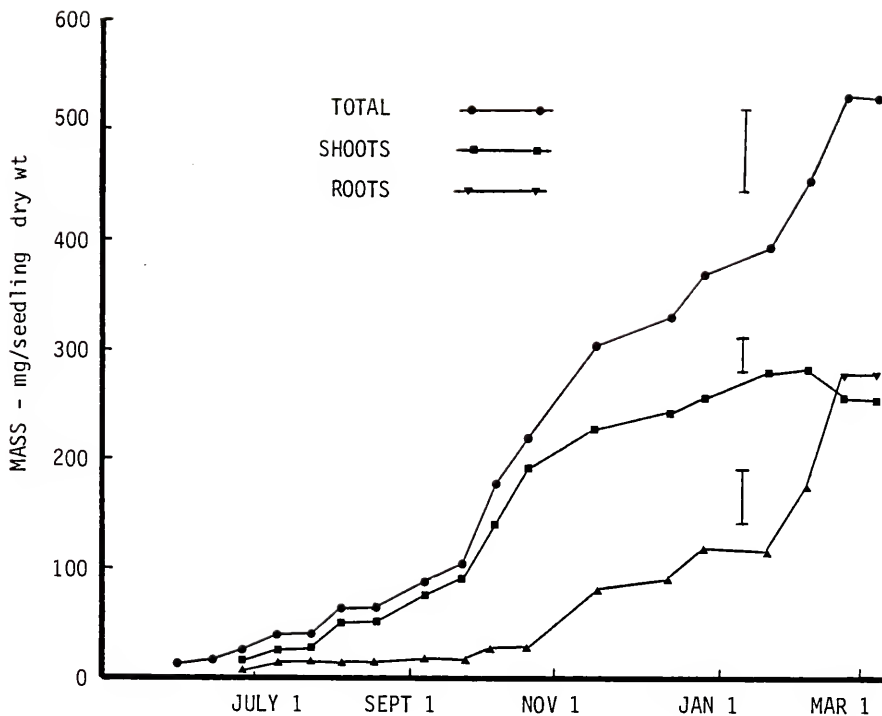


Figure 1-10. Absolute amount of non-structural carbohydrate per seedling. Each point is an average of eight composite samples of 10 seedlings. Bars represent the maximum $S_{\bar{x}}$ for any single point.

The mass of total non-structural carbohydrate per seedling (Figure 1-10) increased over the entire nursery season. This is primarily a function of increasing seedling mass. Sugars compose 89% of the total amount of carbohydrate on October 19, and starch only 11%. This difference gradually diminishes until on February 24 when 48% of the total reserves are sugars and 52% are starches.

Proportionality of Dry Weight Increases

Seasonal variability in photosynthate allocation is indicated in Table 1-1. The nursery season has been divided into four equal periods of 5 weeks each. Dry weight increases of shoots were largest in the late summer, while the roots added more dry weight in the winter. The reduced rate of growth during the period of October 19 to December 15 is most likely related to the low rainfall, normal for this time of year (Dohrenwend 1978), coupled with the practice of withholding irrigation at this time.

Seasonal variability of TNC increase per seedling followed that of dry weight. The greatest increase in weight and carbohydrate reserves of the shoots took place between July 22 and October 4 (Table 1-1), whereas the greatest increase in root weight and carbohydrate content occurred between December 15 and February 24.

Table 1-1. Periodic changes in dry weight and total non-structural carbohydrate (TNC) per seedling (R = roots, S = shoots, T = total).

Sample period	<u>Dry weight increase</u>			<u>TNC increase</u>			<u>ΔTNC/Δ dry weight</u>		
	R	S	T	R	S	T	R	S	T
	(mg)			(mg)			(mg)		
May 27 - July 22	129	497	626	5	27	32	4	5	5
July 22 - Oct 4	329	1523	1852	27	165	192	8	11	10
Oct 4 - Dec 15	198	530	728	45	114	159	23	22	22
Dec 15 - Feb 24	562	181	743	135	66	201	24	36	27

The percentage of total dry weight increase attributable to non-structural carbohydrates increased from 5% in the early summer to 27% during the winter. Thus, during the late fall and throughout winter, the seedlings were allocating ever-increasing amounts of photosynthate to storage instead of growth. Regardless of season, the proportion of mass increase attributable to reserve carbohydrates is roughly similar for roots and shoots, with a maximum difference in the final period (24% vs. 36%). This is unexpected as the root is normally thought of as a storage organ that would accumulate carbohydrates at a faster rate than it would form new cell wall material.

Discussion

The results of morphological analysis demonstrate that the relative growth rates of roots and shoots changed according to season. Fertilization undoubtedly influenced the patterns of growth by stimulating shoot development during the summer. The rapid summer growth differed from Huberman's (1940) early finding with slash pine in which seedlings increased in height at a more or less constant rate through the summer and fall and then leveled off at 25 to 30 cm in November and December. The present study also found height to level off at 25 to 30 cm, but this occurred in September. Presumably, these results are influenced by

the fertilization practices not employed when Huberman did his work. Climate may have been a factor also, as the former study was done in central Louisiana.

The summer applications of nitrogen increased top growth more than root growth. The regression coefficient of 1.01 from the allometric analysis, also known as the coefficient of allometric growth (Leopold and Kreideman 1975), is high when compared to a previously reported value of .75 for potted loblolly pine (Ledig et al. 1970), indicating a greater shoot weight accumulation relative to roots during the June through October period.

The present study, as well as others (Huberman 1940, Perry 1971), revealed substantial dry weight increases, presumably due to photosynthesis, during the winter for southern pines. Furthermore, 70 to 80% of the seedlings sampled for this study in December, January, and February had non-dormant terminal buds, with a minimum of 57% on February 10. The lack of a resting terminal bud, however, has been shown not to be detrimental to outplanting survival (Wakeley 1954).

The higher sugar concentrations during fall and winter presumably relate to cold weather protection (Aronsson and Ingestad 1976, Kaurin et al. 1981). Increased sugar concentration, primarily sucrose, in response to lower temperatures, is one of the most important physiological changes occurring during the development of cold hardiness (Kramer and Kozlowski 1979).

Root starch concentrations began to increase in November and continued to increase throughout the winter, with no certain peak (Figure 1-8). This is in contrast to the winter increases of seedling root starch concentrations reported for Douglas-fir (Krueger and Trappe 1967), which increased from 0.5% in late February to 8% in middle March. Thereafter, root starch gradually declined through the growing season. In the present study, slash pine seedlings began starch accumulation earlier and attained considerably higher concentrations. Without samples later in March, it cannot be determined whether the small decline from February 24 to March 8 was the beginning of a downward trend.

The winter starch concentrations reported here are similar to those of Barnard et al. (1981). These authors had sampled the same nursery 4 years earlier and found average root starch concentrations of 7.6% in December and 14.5% in February. Samples from other Florida nurseries indicated similar increases over winter. One nursery had a much smaller increase, from 5.4% in December to only 7.6% in February, whereas three other nurseries averaged about 12.5% in February.

While caution is advisable when only 1 year's data are available, the results of the present study suggest that in terms of carbohydrate reserves, seedling quality increases throughout the lifting season. This fact may have a direct effect upon the storability of seedlings as the season progresses, inasmuch as several authors have shown that carbohydrate reserves decrease

during storage (Hellmers 1962, Ronco 1973, McCracken 1979, Ritchie 1982). If slash pine seedlings are lifted in late January or February, their ability to survive long-term storage should be better than if lifted in November or December.

How the increased reserves would relate to root growth capacity upon outplanting is difficult to ascertain. If the winter reserves can be utilized for root regeneration, then obviously RGC will be enhanced. Root regeneration is a complex phenomenon, however, and is influenced by the physiological controls associated with winter dormancy. For example, Ritchie (1982) found RGC of Douglas-fir seedlings independent of carbohydrate concentration. Physiological controls such as chilling requirements and photoperiod response are involved (Lavender 1964, Stone and Jenkinson 1971). Therefore, even though carbohydrate may be available for root regeneration, hormonal controls may not allow the formation and/or expansion of primordia until specific environmental conditions are met.

Slash pine does not show the same degree of temperature and photoperiod control (Kaufmann 1977, Fisher 1981) as compared with more temperate species, such as Douglas-fir. The mild winters of its native range presumably preclude the necessity for such controls, and the species can physiologically respond to the sunny days which frequent northern Florida in December through February. This was demonstrated by the sustained dry weight increases throughout the winter and the lack of a well formed dormant terminal bud.

The data reveal intense root activity during the winter (Table 1-1). Therefore, no physiological "switch" needs to be turned on to initiate root growth after outplanting. It is plausible that root growth immediately after planting is supported and strengthened by reserves. The amount of this support required probably depends upon how quickly the seedling can re-establish normal water relations and the photosynthetic process.

CHAPTER II CHANGES IN SEEDLING MORPHOLOGY AND CARBOHYDRATE RESERVES AFTER OUTPLANTING

Introduction

Non-structural carbohydrates contribute to plant growth via three basic metabolic functions: (1) as a substrate for respiration; (2) as the basic component of structural polysaccharides; and (3) as carbon skeletons for other essential organic compounds (Priestly 1962). The ultimate source of carbohydrates is photosynthesis, but, in the case of outplanted seedlings, the photosynthetic process is interrupted by root loss resulting from lifting and trimming, perhaps by cold storage, by transport, handling and planting, and finally, by an almost inevitable period of post-planting moisture stress. Furthermore, the effects of outplanting on photosynthetic production would be further prolonged and intensified by dry field conditions.

Under these circumstances, reserve carbohydrates may be used for basic metabolism, particularly respiration and cell wall formation. Stored reserves can therefore directly support the root

extension necessary for survival after outplanting. If it could be shown that seedling TNC decreases substantially upon outplanting, then a strong case would be made for their contribution to survival.

A study to follow the changes in seedling morphology and TNC after outplanting was combined with a study of undercutting in the nursery. Seedlings subjected to different intensities of undercutting in the nursery have been shown to have significant morphological (Rook 1971, Tanaka et al. 1976) and physiological differences (Rook 1971) when compared to intact controls. Moreover, the differences resulting from undercutting have been associated with improved seedling performance upon outplanting (Shoulders 1963, Rook 1971, Tanaka et al. 1976). Thus, undercutting was expected to produce seedlings with different morphological and physiological qualities in which the contribution of TNC to performance after outplanting could be examined.

Materials And Methods

Nursery

The nursery phase of the experiment was carried out in the same nursery described in Chapter I. Seed source, location, and

cultural treatments were also identical. The nursery experimental design was a randomized complete block with four treatments replicated four times. The four treatments were as follows:

Treatment C- Control, no undercutting prior to lifting;

Treatment 1- undercut once, 2 weeks prior to lifting;

Treatment 2- undercut twice, at 6 and 2 weeks before
lifting;

Treatment 3- undercut four times, at 8, 6, 4, and 2
weeks before lifting.

Undercutting was done with a tractor-drawn bar that cut roots about 15 cm below the bed surface. The bar lifted the bed about 2 cm as it worked and therefore had a "wrenching" effect. Each treatment plot was 19.8 m long and one bed wide (Figure 2-1).

The first undercutting was on November 9, 1982, 8 weeks before lifting on January 4, 1983. All treatments were undercut at the time of lifting in accordance with standard nursery practice. This made the total number of undercuttings to be 1, 2, 3, and 5 times, for the control and treatments 1, 2, and 3, respectively.

At this time, each treatment plot was sampled by hand, pulling 30 seedlings from each of four equally spaced subplots (Figure 2-1). Each subplot covered a different two rows of the

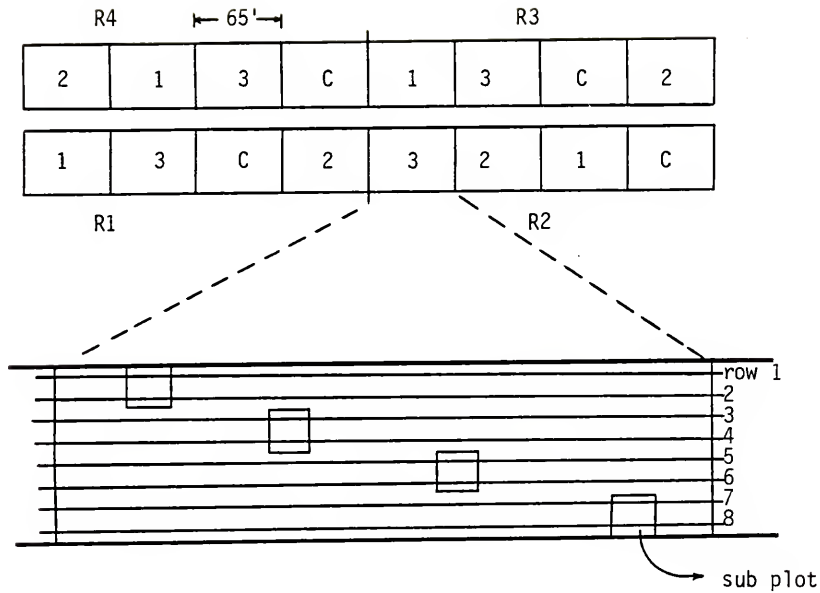


Figure 2-1. Archer nursery root pruning study indicating replication, treatment, and sample plot location

- C - Control, no root pruning;
- 1 - Root pruned 2 weeks prior to lifting;
- 2 - Root pruned 6 and 2 weeks prior to lifting;
- 3 - Root pruned 8, 6, 4, and 2 weeks prior to lifting.

bed and seedlings were sampled from both rows. Thus, there were 120 seedlings per treatment plot, representing all rows. The four subplot samples were mixed. Twenty samples were separated for laboratory analysis. The remaining 100 trees from each plot were placed in KP bags with hydromulch and stored at 5°C until outplanting occurred 2 days later.

Field

The field experiment was hand planted with dibbles on January 6. The design was a randomized complete block with four replications. Nursery replications were continued in the field. Each plot was a row of 80 seedlings spaced 0.6 m (2 feet) within rows and 3.6 m (12 feet) between rows. The soil was a moderately deep, well-drained loamy sand of the Jonesville series. The soil was moist at the time of planting and the weather cool, partly cloudy, and windy. The site had been a mature slash pine plantation which had been harvested in 1981 then drum chopped, raked, burned and bedded during the summer of 1982. Weed control was generally good.

After outplanting, five seedlings per row were sampled every 2 weeks for 12 weeks. The seedlings were carefully excavated, attempting to leave the root system intact. Each five tree sample was used for laboratory analysis. After the 12 week period, the remaining 50 trees per row were tallied for survival at 13 weeks and survival and height at 1 year. Daily rainfall was measured at a fire tower located 2 miles east of the planting site.

Laboratory

Laboratory procedure for all samples was identical to that of Chapter I, with two exceptions. At each sampling time, the unsuberized roots of each seedling were cut off to obtain a total dry weight of new roots. Also, the percent moisture of all samples was determined after drying for 1 hour at 70°C, and 23 hours at 105°C. Root/shoot ratio was calculated on a dry weight basis.

Statistical Analysis

The General Linear Model procedure of the Statistical Analysis System (SAS) (Freund and Littell 1981) was employed for comparisons between replications, treatments, and sample times. A split plot design was used to compare times, treatments, and times x treatment interaction during the 12 week sampling period after outplanting (Table 2-1). A randomized complete block design was used for comparing treatments at the time of lifting. The "Contrast" statement of SAS was used to make orthogonal comparisons between treatment means and time means for linear, quadratic, and cubic models.

Post-outplanting field samples of the undercutting experiment were being taken simultaneously with those of the seedling development study presented in Chapter I. Therefore, the control treatment of the undercutting experiment was directly comparable to the results presented in Chapter I inasmuch as they were from adjacent nursery areas and received identical nursery care up to

Table 2-1. Analysis of variance design.

A. For time of lifting

<u>Source of variation</u>	<u>df</u>
Replications	3
Treatments	3
Error	9
	<hr/>
Total	15

B. For all sampling times

<u>Source of variation</u>	<u>df</u>
Replication	3
Treatments	3
Error A	9
Time	6
Time x treatment	18
Error B	72
	<hr/>
Total	111

the time of lifting. Both morphological and carbohydrate values of the two experiments were compared for the period of time when they were sampled simultaneously.

Furthermore, when there were no statistical differences among undercutting treatments, all treatments were averaged to increase the number of individuals used to compare outplanted seedlings to nursery seedlings.

Due to a freezer malfunction, samples taken at the time of lifting were unavailable for the purpose of carbohydrate determination. The carbohydrate levels at lifting time can be estimated by using data from the December 28 and January 24 values presented in Chapter I. These values were not used, however, in any statistical analysis of the present experiment.

Results

Morphological Characteristics at Lifting

Height, diameter at root collar (DRC), and root/shoot ratio--characteristics often used to assess seedling quality--were not statistically different among any of the treatments at the time of lifting (Table 2-2).

The control seedlings were significantly lower in both fresh and dry weight than undercut seedlings. Treatment 1 seedlings averaged 599 mg more dry weight than the controls. This is unexpected inasmuch as treatment 1 was root pruned only one time, 2

Table 2-2. Treatment means for morphological variables measured at the time of lifting (R = root, S = shoot, T = total)

Treatment ^{1/}	Height	DRC	Fresh weight		Dry weight		New roots	R/S ratio	Moisture content			
			R	S	R	S			R	S		
cm												
mm												
gm												
-----gm-----												
mg												
----- % -----												
Control	26.4	3.6	1.39	7.32	8.71	.50	2.14	2.64	2.5	.234	178	242
1	26.2	3.8	1.69	8.93	10.62	.62	2.62	3.24	4.3	.236	178	240
2	25.7	3.8	1.71	8.44	10.15	.61	2.50	3.12	5.2	.242	186	236
3	25.2	3.8	1.94	8.48	10.42	.65	2.45	3.10	14.9	.267	196	246
Level of significance ^{2/}	NS	NS	.01	.05	.04	NS	.04	.05	.01	NS	NS	NS
Model			L	Q	L		Q	Q	L			

^{1/} All treatments were undercut at lifting, making the total number of undercuttings for the control and Treatments 1, 2, and 3 to be 1, 2, 3, and 5 times, respectively.

^{2/} Statistical differences between treatments resulting from orthogonal comparisons using the "Contrast" procedure of SAS, General Linear Models. Best Fit Models were either Linear (L) or Quadratic (Q).

weeks prior to lifting. The effect was consistent for all four replications.

No statistically significant difference in root dry weight was found among treatments and the control. There was, however, a significant linear increase in root fresh weight and the number of unsuberized roots with increasing intensity of undercutting (Table 2-2). The undercutting resulted in a profusion of new roots. The most intensively pruned treatment had six times the dry weight of new roots as the control (14.9 mg vs. 2.5 mg).

Morphological Changes after Outplanting

There were no significant interactions between treatment and time after outplanting with the exception of new roots. This fact allows comparison among treatments for combined time averages for all varieties except new roots. For example, the height measurements for all four treatments were combined to increase the sample size for each sampling time after outplanting.

The seedlings increased significantly in height, DRC, and dry weight in the 12 weeks after outplanting (Table 2-3). Although the data for dry weight increase fit a linear model over the 12 weeks (Table 2-3), the actual increase was negligible up to 6 weeks followed by an abrupt surge. The largest increase in height and terminal bud activity began after the eighth week.

Table 2-3. Seedling morphological measurements after outplanting averaged across treatments by sampling date (R = root, S = shoot, T = total).

Weeks after planting	Date	Height cm	DRC mm	Fresh weight		Dry weight		New roots ratio	Moisture content							
				gm		gm			R	S						
				R	S	R	S				R	S				
				gm	gm	gm	gm	mg	%							
0	Jan. 6	25.9	3.7	1.7	8.3	10.0	.60	2.4	3.0	6.7	.25	184	241			
2	Jan. 20	25.7	4.0	1.6	8.4	10.0	.58	2.6	3.2	2.9	.22	185	215			
4	Feb. 3	26.8	4.0	1.6	7.7	9.3	.62	2.7	3.3	1.9	.23	161	183			
6	Feb. 17	26.4	4.0	1.6	6.7	8.3	.65	2.7	3.3	8.4	.24	149	159			
8	Mar. 3	25.9	4.1	2.1	7.3	9.4	.88	3.0	3.9	22.9	.29	138	144			
10	Mar. 17	26.7	4.3	2.6	7.9	10.5	.87	3.0	3.9	42.5	.29	196	162			
12	Mar. 31	28.4	4.3	3.0	8.3	11.3	.91	3.1	4.0	42.5	.29	235	167			
Level of Significance ^{1/}											.001	.001	.001	.001	.001	
Model											L	L	L	L	Q	Q

^{1/} Statistical differences between times resulting from orthogonal comparisons using the "Contrast" procedure of SAS, General Linear Models. Best Fit Models were either Linear (L) or Quadratic (Q).

The dry weight accumulation of outplanted seedlings over the 12 week period was smaller than that of seedlings remaining in the nursery (Figure 2-2). Shoot dry weights of nursery and outplanted seedlings were essentially the same during the 12 week study period. Root dry weight differed considerably between nursery and outplanted seedlings due to an approximate 30% reduction in root dry weight upon lifting and outplanting. As a result, total dry weight of outplanted seedlings was below that of seedlings remaining in the nursery. This difference had disappeared by 8 weeks after planting, indicating that outplanted seedling growth rate was faster than seedlings remaining in the nursery.

While there was no dry weight loss after outplanting, seedlings lost water in both roots and shoots during the first 8 weeks (Figure 2-3). After 8 weeks, average percent moisture increased substantially in shoots and only slightly in roots. Analysis revealed significant differences in moisture content among sample dates using a quadratic model for roots and shoots (Table 2-3).

Seedlings developed significant differences among treatments during the 12 week period after outplanting for several morphological characteristics (Table 2-4). For the most part, this was related to root growth. Nursery undercutting significantly increased root fresh and dry weight, root/shoot ratio, number of new roots, and root moisture content. Root growth increased linearly with increasing intensity of nursery root pruning.

Figure 2-2. Average root, shoot, and total dry weight per seedling after outplanting compared to seedlings remaining in the nursery. Values for nursery seedlings were given in Chapter I. Bars indicate $\pm S_{\bar{x}}$. Shaded bars in the bottom indicate rainfall at the planting site for specific dates.

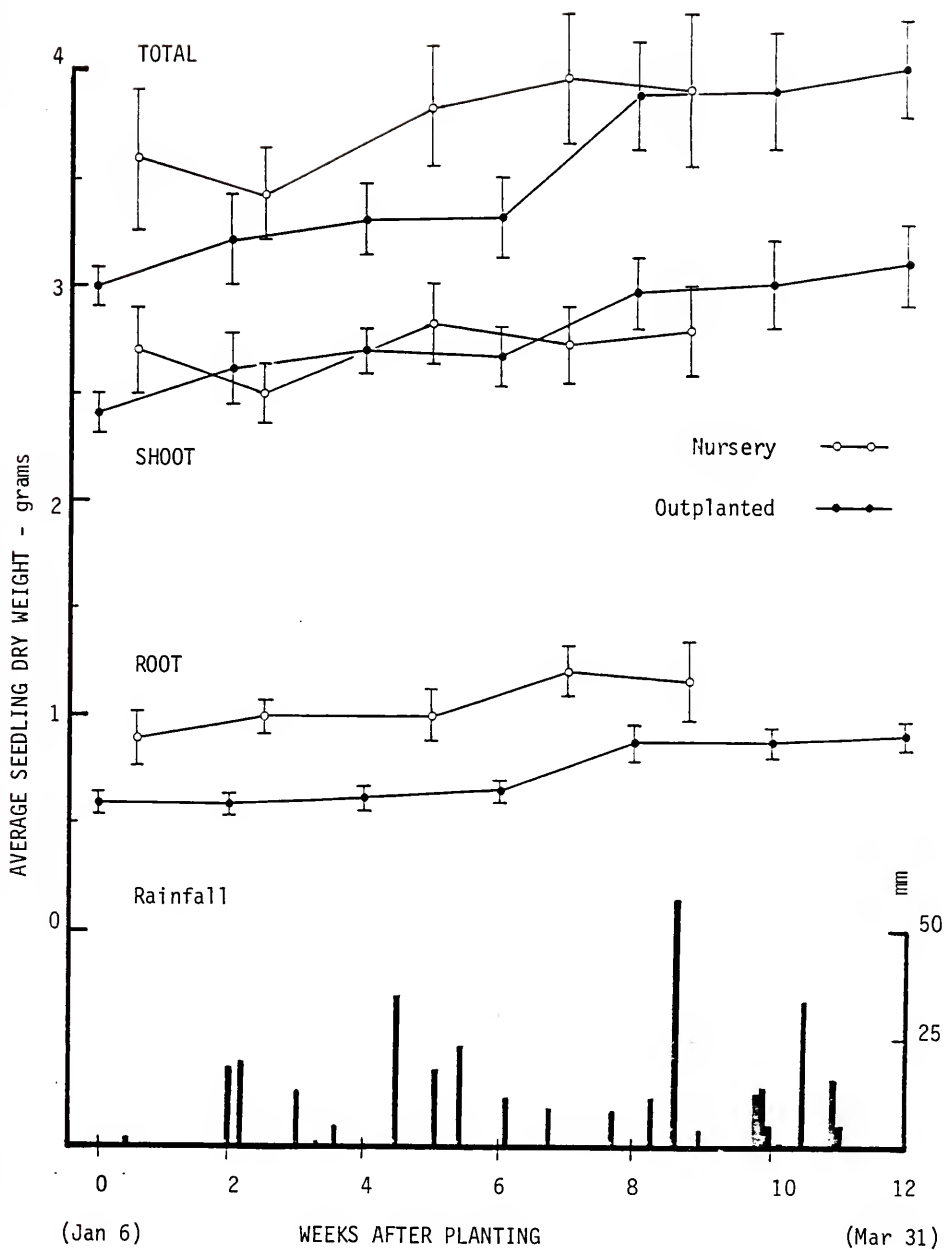


Figure 2-3. Root and shoot moisture content of outplanted seedlings as compared to those remaining in the nursery. Bars indicate $\pm S_{\bar{x}}$. Values for nursery seedlings were presented in Chapter I.

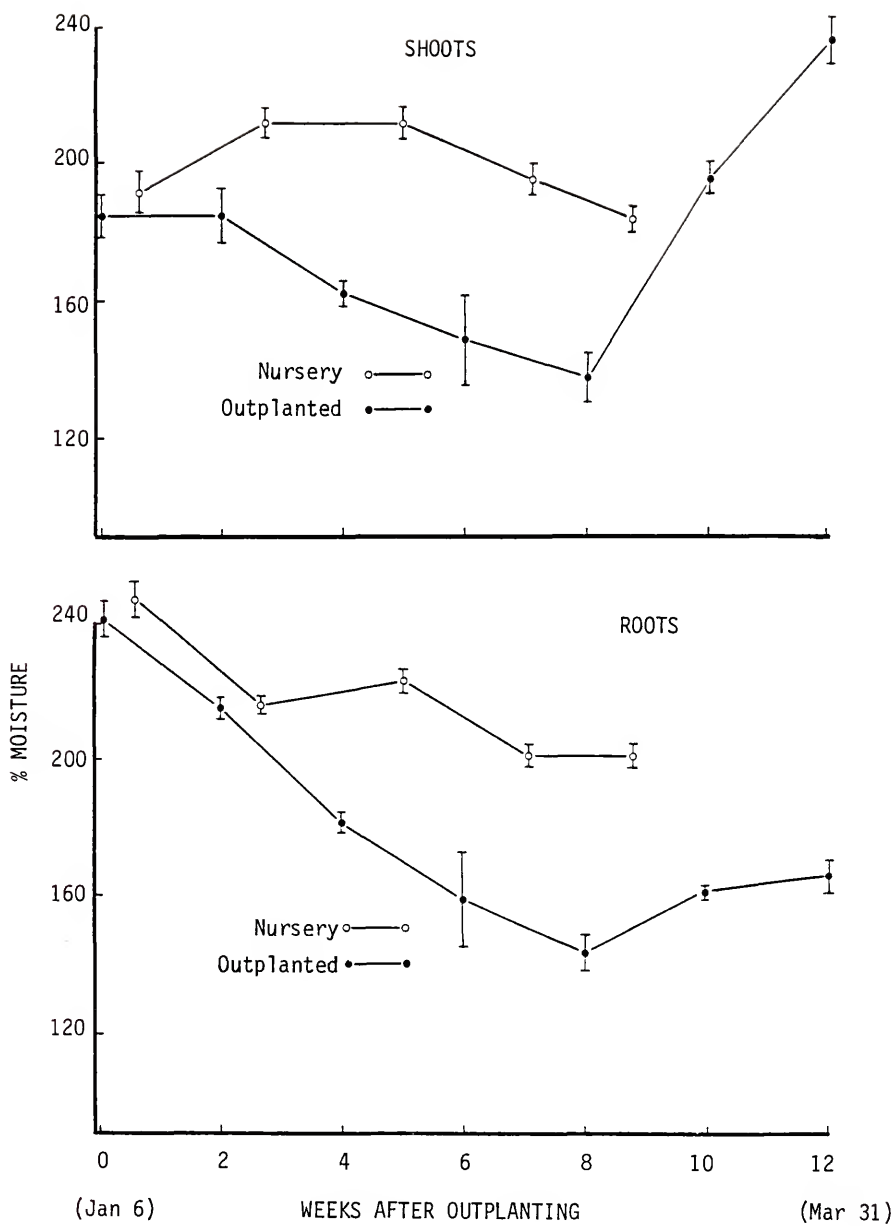


Table 2-4. Treatment means for morphological variables averaged across replications and sampling time 0 through 12 weeks (R = roots, S = shoots, T = total).

Treatment ^{1/}	Height	DRC	Fresh weight			Dry weight			New roots	R/S ratio	Moisture content	
			R S T			R S T					R	S
			----- gm -----			----- gm -----						
	cm	mm							mg		---- % ----	
Control	27.0	3.9	1.66	7.11	8.77	.59	2.55	3.14	12.0	.229	180	181
1	27.0	4.1	1.94	8.14	10.09	.73	2.92	3.65	12.7	.251	166	180
2	26.1	4.2	2.16	8.29	10.45	.77	2.92	3.69	22.6	.257	178	187
3	26.0	4.1	2.45	7.66	10.11	.83	2.79	3.62	25.8	.297	189	178
Level of significance ^{2/}												
	NS	NS	.02	.03	NS	.02	NS	NS	.01	.03	.01	NS
Model			L	Q		L			L	L	L	

^{1/} All treatments were undercut at lifting, making the total number of undercuttings for the control and treatments 1, 2, and 3 to be 1, 2, 3, and 5 times, respectively.

^{2/} Statistical differences between treatments resulting from orthogonal comparisons using the "Contrast" procedure of SAS, General Linear Models. Best Fit Models were either Linear (L) or Quadratic (Q).

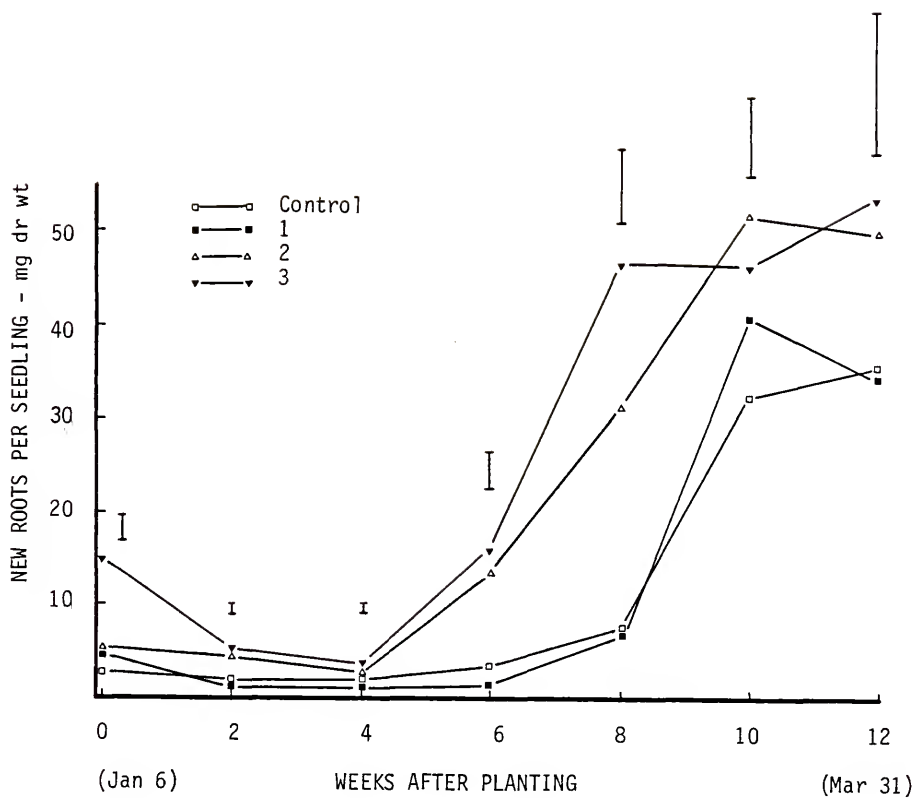


Figure 2-4. Average dry weight of new roots per seedling after outplanting. Bars indicate the largest $S_{\bar{x}}$ for any one sample date.

The only treatment x time interaction concerned new root production. Treatment 2 and 3 added new roots faster and earlier than either treatment 1 or the control (Figure 2-4). The growth of new roots began after four weeks in the field. This is immediately before the surge in total dry weight occurring after 6 weeks (Figure 2-2). Also, new root growth occurred even while the overall root moisture content declined (Figure 2-3).

Changes in Carbohydrate Reserves after Planting

Analysis revealed no interaction between time and treatment for any carbohydrate variable, either concentrations or amounts. This allowed comparison among times using combined treatment averages and comparisons among treatments using combined time averages.

Although mean total carbohydrate content differed appreciably with treatment, only one of the differences in carbohydrate concentration or content was statistically significant (Table 2-5). This may be a result of the relatively small sampling unit (five seedlings) or the relatively variable field environment. Only root starch mass was found to vary significantly by treatment. This relationship was linear, with amounts of root starch increasing with intensity of nursery undercutting.

Carbohydrate reserves changed significantly with sampling date (Table 2-6). From January 20 (2 weeks after planting) to March 31 (12 weeks after planting), sugar concentrations

Table 2-5. Carbohydrate values by treatment averaged across sampling times after outplanting (R = root, S = shoot, T = total).

Treatment	1/ mg/g dry wt	Concentration						Total	Mass						Total
		Sugar			Starch				Sugar			Starch			
		R	S	T	R	S	T		R	S	T	R	S	T	
		mg/seedling													
Control	57	78	67	79	41	59	127	34	205	237	47	108	155	394	
1	58	79	69	66	33	50	119	43	236	279	49	100	149	428	
2	57	84	70	87	43	65	135	45	250	295	69	130	199	494	
3	56	81	68	92	42	67	135	47	230	277	79	120	199	476	
Level of significance ^{2/} Model	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	.03 L	NS	NS	

1/ All treatments were undercut at lifting, making the total number of undercuttings for the control and treatments 1, 2, and 3, to be 1, 2, 3, and 5 times, respectively.

2/ Statistical differences between treatments resulting from orthogonal comparisons using the "Con-
trast" procedure of SAS, General Linear Models. Best Fit Models were either Linear (L) or Quadratic (Q).

Table 2-6. Average values for carbohydrate variables for each sampling date averaged across treatments (R = roots, S = shoots, T = total).

Weeks after planting	Date	Concentration						Mass					
		Sugar			Starch			Sugar			Starch		
		R	S	T	R	S	T	R	S	T	R	S	T
----- mg/g dry wt -----													
0	Jan 4 ^{1/}	--	--	--	72	10	41	--	--	--	43	25	78
2	Jan 20	72	82	77	40	10	25	--	42	215	24	26	50
4	Feb 3	66	92	79	60	13	37	116	41	249	38	35	73
6	Feb 17	63	87	75	78	23	51	126	40	237	277	53	61
8	Mar 3	54	84	69	102	44	73	142	48	250	298	92	130
10	Mar 17	44	74	59	100	66	83	142	39	227	266	87	203
12	Mar 31	43	64	54	104	86	95	149	40	197	237	99	281
----- mg/seedling-----													
Level of Significance	2/	.01	.01	.01	.01	.01	.01	.01	NS	.01	.01	.01	.01
Model		L	L	L	L	L	L	L	Q	Q	L	L	L

1/ Samples taken on January 4 are unreliable due to a freezer malfunction. The analytical values were not included in comparison of means, but the starch concentrations are reported because they are similar to those of nursery seedlings sampled at this time (Chapter I).

2/ Statistical differences between treatments resulting from orthogonal comparisons using the "Con-
trast" procedure of SAS, General Linear Models. Best Fit Models were either Linear (L) or Quadratic (Q).

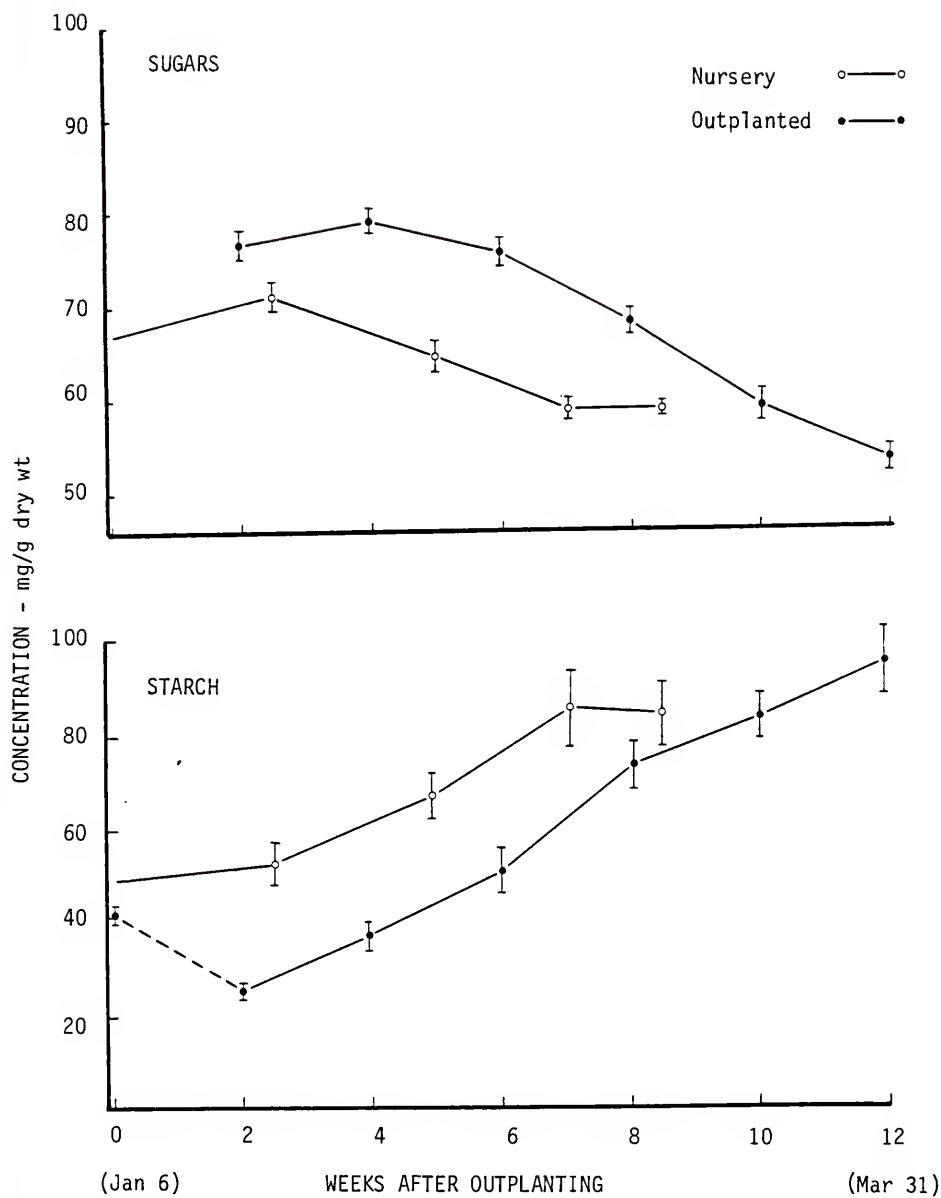
decreased while starch concentrations increased. The only exception to this trend was shoot sugar concentrations which increased from the second to fourth week after outplanting (January 20 to February 3), then began to decline.

The values determined for root and shoot starch concentrations at time zero are unreliable due to sample thawing after a freezer malfunction. These values are shown in Table 2-6, however, because they are similar to those of nursery samples taken near the same time (Figure 2-6). If they are accurate, they would indicate a 44% decrease in root starch concentrations during the first 2 weeks after outplanting.

The changes over time in carbohydrate concentrations after outplanting were remarkably similar to the changes in nursery seedlings during the same period (Figure 2-5, 2-6, 2-7). Starch concentration increased and sugar concentration decreased in both. Generally, reserve concentrations of outplanted seedlings followed the same seasonal trends as were encountered in seedlings remaining in the nursery.

There were, however, two important differences between the data sets. First, starch concentrations of outplanted seedlings were lower than those of nursery seedlings during the first 8 weeks, whereas sugar concentrations were higher (Figure 2-5).

Figure 2-5. Average sugar and starch concentration per seedling after outplanting, compared to those of seedlings remaining in the nursery. Bars represent $\pm S_{\bar{x}}$. Values for nursery seedlings were presented in Chapter I; lines here have been extended to values for December 28. The value for starch concentration of Time 0 is actual analysis result, but may be erroneous (see text).



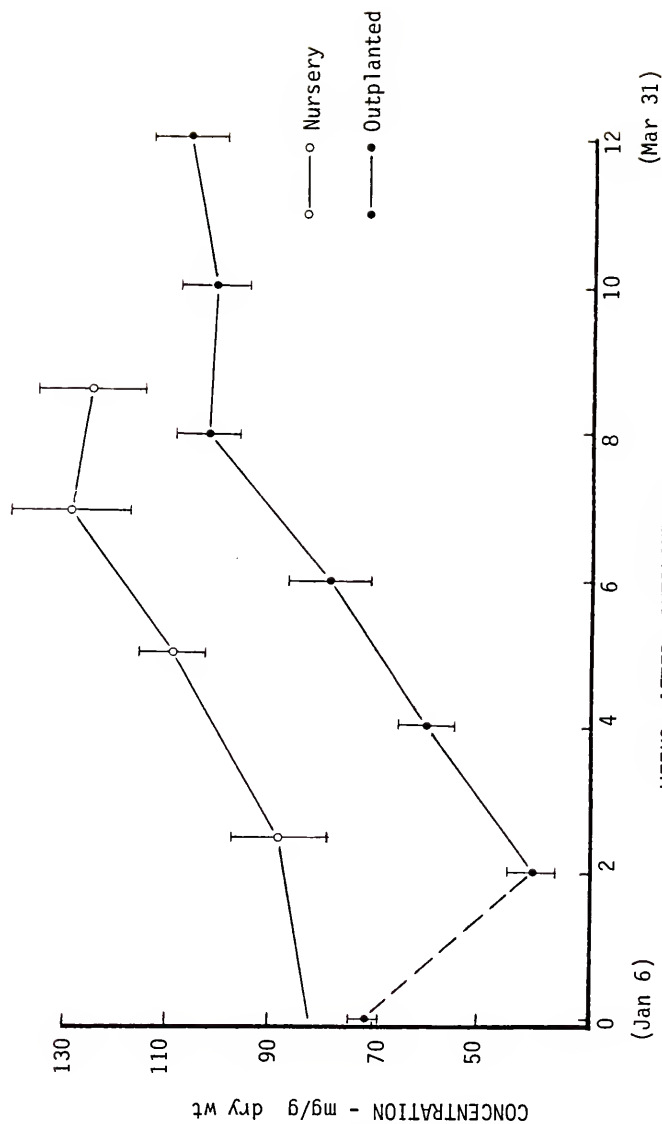
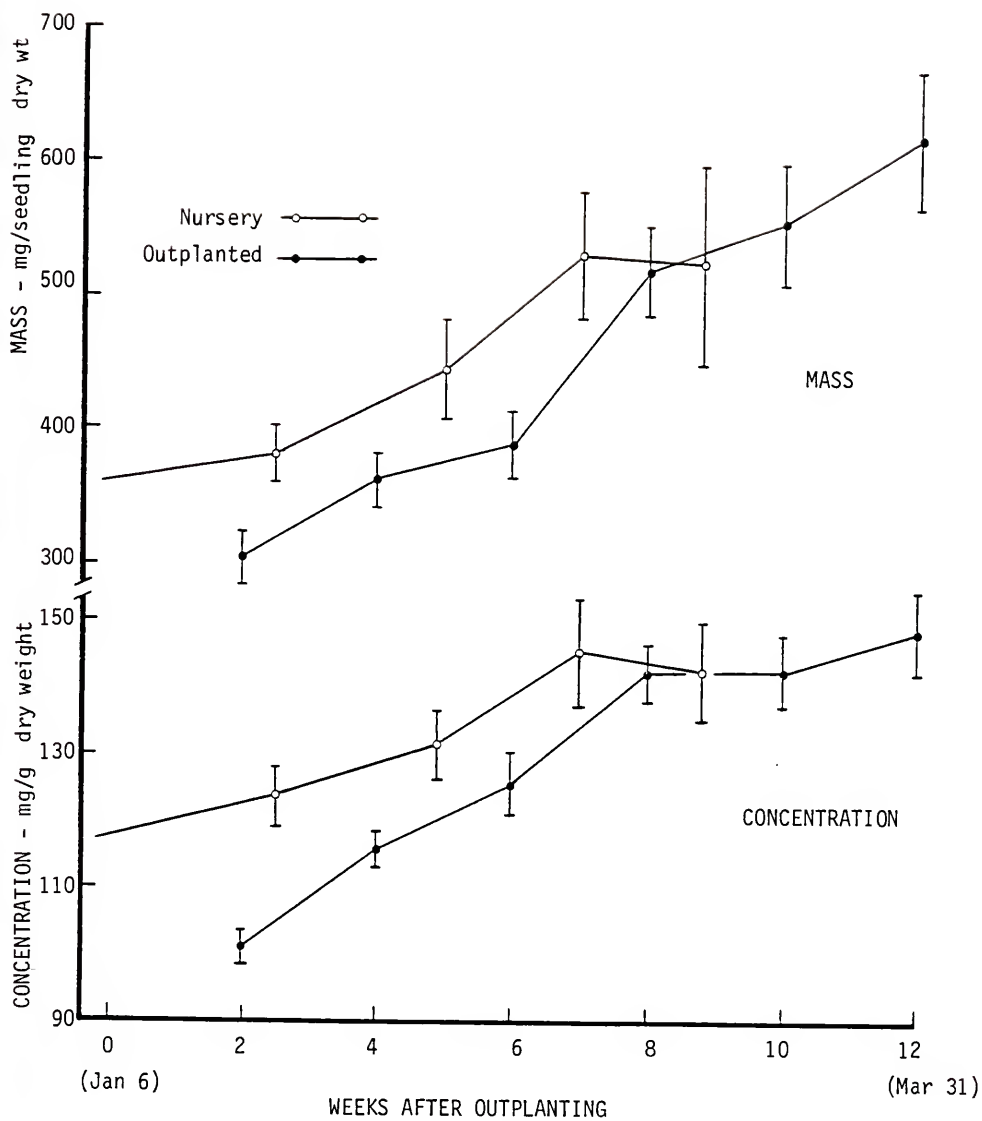


Figure 2-6. Average root starch concentration after outplanting compared to that of seedlings remaining in the nursery. Bars represent $\pm S_x$. Values for nursery seedlings were presented in Chapter I; the slope above has been extended to the actual value on December 28. The starch concentration for Time 0 is as actually determined, but may be erroneous (see text).

Figure 2-7. Average carbohydrate concentrations and absolute amounts for whole seedlings after outplanting compared to those of seedlings remaining in the nursery. Bars represent $\pm S_{\bar{x}}$. Values for nursery samples were presented in Chapter I; lines here have been extended to values for December 28.



Second, total carbohydrate reserves of outplanted seedlings declined in both concentration and amount (Figure 2-7) during the first 2 weeks after planting. As noted before, a 44% decrease in root starch concentrations may have occurred in the roots during the first 2 weeks after planting. The loss in reserves was in part related to the 30% reduction in root mass upon lifting. The field-planted seedlings caught up to and surpassed nursery seedlings with respect to total reserves after 8 weeks.

Survival and Height at 1 Year

Height growth and survival after a year in the field differed significantly among treatments. Survival of seedlings from treatment 3 was 96% versus 72% for the controls. The relationship between survival and the number of nursery undercuttings was curvilinear, with an R^2 of .56 (Figure 2-8). An arc sine transformation of survival percent increased the R^2 to .59. Thus, increasing the frequency of nursery root pruning significantly increased field survival.

As noted, seedling height at the time of lifting did not vary among treatments (Table 2-2). Yet after 1 year in the field, control seedlings averaged 33.8 cm height and treatment 3 averaged 39.7 cm height. Frequency of root pruning also improved

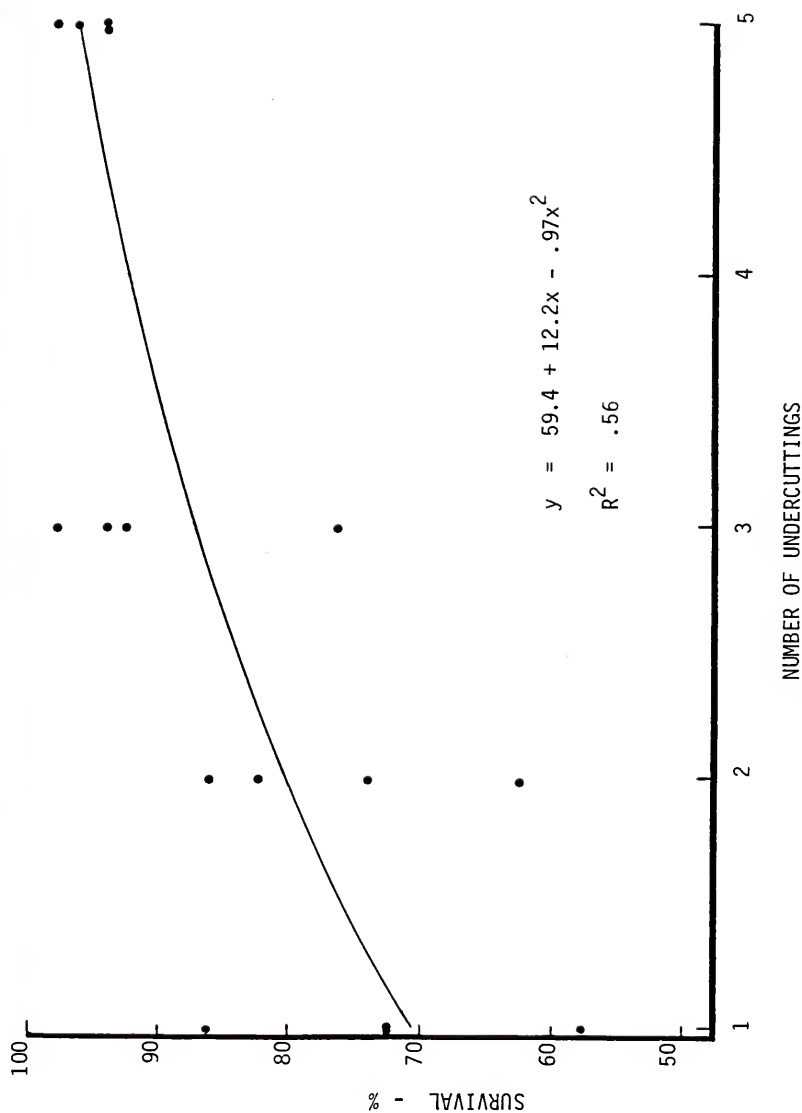


Figure 2-8. The relationship of survival at 1 year to the number of undercuttings prior to outplanting. (One undercutting occurred at lifting, the others at 2, 4, 6, and 8 weeks before lifting.) The regression equation was significant at the .01 level.

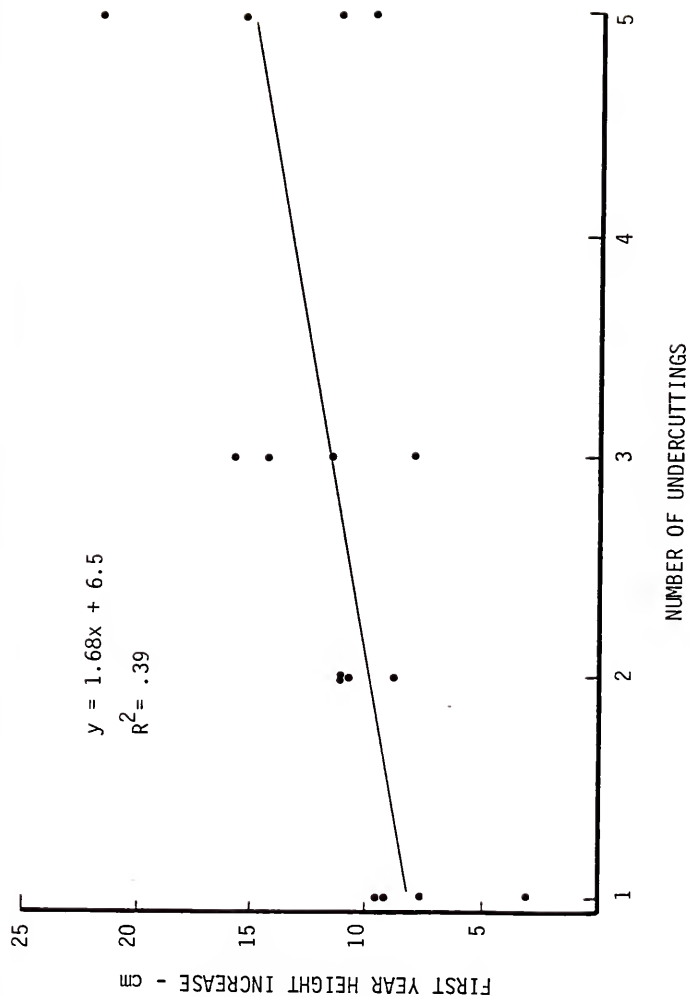


Figure 2-9. The relationship of first year height growth to the number of undercuttings prior to out-planting. (One undercutting occurred at lifting, the others at 2, 4, 6, and 8 weeks before lifting.) The regression equation was significant at the .01 level.

first year height increment, $R^2 = .39$ (Figure 2-9). The low R^2 indicates considerable variability in treatment response, however.

Correlations between field performance and morphological or carbohydrate reserve variables were tested using a large number of linear models. Table 2-7 presents only the models which tested significantly, i.e., whether individual model coefficients are equal to zero (Freund and Littell 1981). Even so, the R^2 values resulting from these models were all low. The maximum amount of variability accounted for was 48% in the case of survival vs. new root mass (Figure 2-10) and 50% in the case of new root mass vs. total carbohydrate concentration (Figure 2-11). The scatter of points is considerable in both cases even though a definite linear response is discernable.

Figure 2-11 indicates a 1.1% increase in survival per additional milligram of new roots in the 1-12 week period after outplanting. The amount of new roots per seedling is, in turn, related to the amount of carbohydrate reserves (Figure 2-11). Morphological characteristics normally used as seedling quality indicators, such as height, DRC, and root/shoot ratio, were poor predictors of field performance.

Table 2-7. Linear Regression Models for which the test $B \neq 0$ proved significant at $\alpha = .05$ (Freund and Littell 1981).

-
1. Survival = $1.12(x) + 75.8$ $R^2 = .25$
 where x = mg of new roots per seedling at lifting.

 2. Survival = $1.1(x) + 61.2$ $R^2 = .48$
 where x = average mg of new roots per seedling
 2-12 weeks after outplanting.

 3. Survival = $.51(x) + 18.1$ $R^2 = .28$
 where x = average total carbohydrate concentration per
 seedling 2-12 weeks after outplanting.

 4. Increment = $.28(x) + 5.5$ $R^2 = .31$
 where increment is the first year's height growth,
 and x = average mg of new roots per seedling 2-12 weeks
 after outplanting.

 5. New Roots = $.43(x) - 35$ $R^2 = .50$
 where new roots = average mg of new roots per seedling
 2-12 weeks after outplanting
 and x = average total carbohydrate concentration per
 seedling 2-12 weeks after outplanting.
-

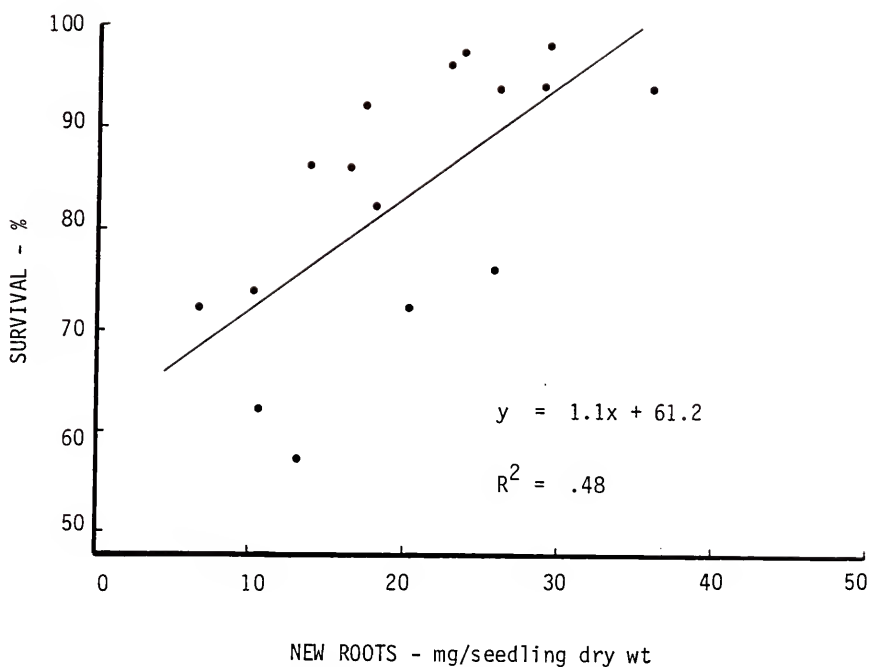


Figure 2-10. The relationship of survival at 1 year after outplanting to average mass of new roots per seedling during the first 12 weeks after outplanting. Each point is an average of 24 composite samples of five seedlings and includes six sampling dates.

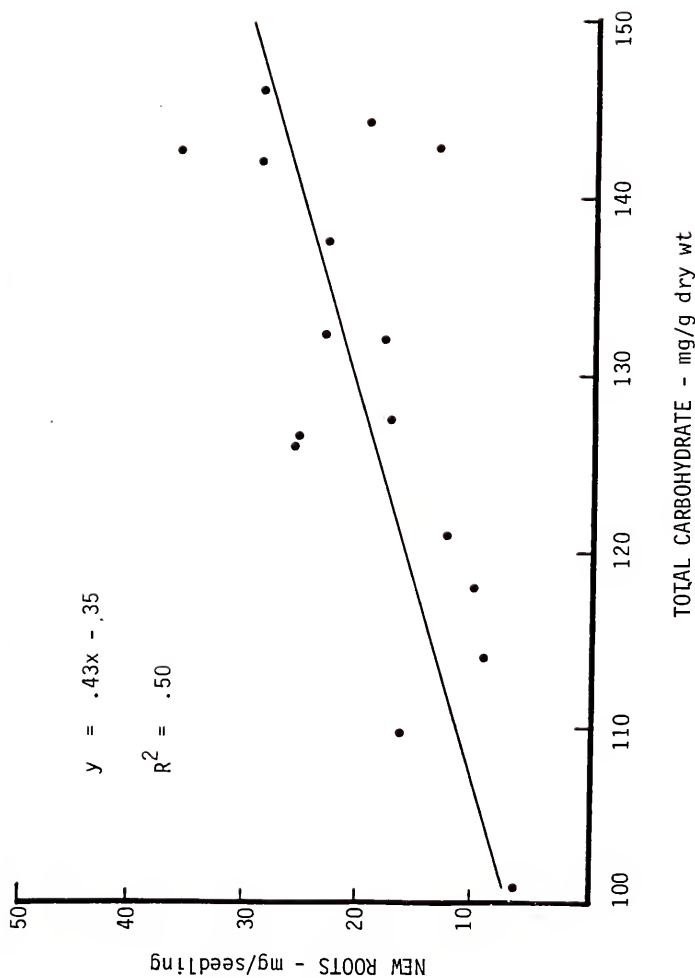


Figure 2-11. The relationship of average new root mass per seedling during the 2-12 week period after outplanting to total non-structural carbohydrate concentration during the same period. Each point is an average of 24 composite samples of five seedlings and includes six sampling dates. The regression line was significant at the .01 level.

Discussion

The lack of morphological variation between treatments at the time of lifting was due to two factors: (1) the treatments were applied in November-December when top growth had slowed (Chapter I); and (2) the 8 week time lapse was not sufficient enough for statistically significant differences to develop. Studies that have found significant morphological variation due to undercutting involved actively growing seedlings and treatments applied over a several month period (Rook 1971, Tanaka et al. 1976). In fact, radiata pine seedlings must be root wrenched when they are actively growing to achieve the desired morphological and physiological modifications (Rook 1971).

Root dry weight was unaffected by the intensity of root pruning (Table 2-2), which concurs with studies of radiata pine (Rook 1971) and loblolly pine (Tanaka et al. 1976). In the present study, the form of roots was modified rather than total weight. November and December is a period when seedlings were actively adding root weight (Chapter I). In undercut seedlings this increase occurred on 70% of the original root mass, inasmuch as 30% had been lost at the first undercutting (Figure 2-2). The more intensive root pruning treatment distributed this increase among a larger number of new roots (Table 2-2). The result of the various undercutting intensities was to produce seedlings of similar dry weight but different root morphologies.

Nursery root pruning caused a profusion of new roots. Root auxin and cytokinin activity are increased by root pruning (Carlson and Larson 1977). It is the specific balance of these two hormones that controls root primordia initiation (Altman and Wareing 1975). Undercutting produced more new roots and root primordia which could expand upon outplanting. The effect of nursery undercutting continued in the field where intensively undercut seedlings produced new roots faster and in greater quantities than controls (Figure 2-4).

Morphological and physiological development after outplanting closely followed that of undisturbed seedlings in the nursery. Apparently, the degree of "transplant shock" experienced by the seedlings in this experiment was not great. The results clearly show that growth can begin shortly after planting. The large dry weight increases between the fourth and sixth week after planting (Figure 2-2) indicate that photosynthetic capacity must have been restored. In fact, increases in TNC indicate that net photosynthesis began soon after the second week in the field. There was only 2.5 mm of rainfall during the first 2 weeks after planting. The increase in TNC after 2 weeks in the field coincided with several good rains (Figure 2-7).

Caution is advisable when interpreting results from a single year study. Lower rainfall or antecedent soil moisture might have limited the ability of seedlings to re-initiate carbon assimilation. Furthermore, lower soil temperature might well have slowed root expansion (Nambiar 1979).

The seedlings did not put out roots at the expense of shoots. Both roots and shoots added dry weight at the same rate they would have in the nursery. One might expect the plant to direct photoassimilates to restore root systems damaged as a result of the lifting and planting process. It has been shown that undercut seedlings may translocate a larger proportion of photoassimilated ^{14}C to their roots than untreated controls (Rook 1971, Bacon and Bachelard 1978). Such redirection to roots did not occur after outplanting in the present study. In fact, when dry weight of outplanted seedlings equaled that of nursery seedlings at about 8 weeks after planting, it was due to the large increases in shoot mass, inasmuch as root mass was still less than that of nursery seedlings (Figure 2-2). During the 12 week period of this study, root dry weight increased by 0.39 g, while shoot dry weight increased by 0.70 g (Table 2-3).

The results of this study concur with the hypothesis that root growth potential is a good indicator of field performance (Sutton 1980) and is a better indicator than the morphological characteristics generally used for grading seedlings. The amount of new roots found on seedlings during the 2 to 12 week period after outplanting correlated with both survival and height increment (Table 2-7). No morphological variable, including root/shoot ratio, showed any correlation with performance.

A certain amount of the unaccounted variability in the regression of first year height increment and new roots ($R^2 = .48$), is a result of site conditions. The experimental area covered 0.17 ha (.44 ac) and subjected seedlings to a range of microsites, arising from varying degrees of vegetative competition, logging debris, and water retention. Furthermore, the soil is sandy and well-drained. Bedding was a result of standard operating procedure rather than a site specific recommendation. Bedding dry sites may in fact decrease first year height increment (Broerman et al. 1981). Water stress, especially during the late summer, could have caused the relatively small seedling height increment during the first year.

It cannot be concluded from this study that RGP is dependent upon carbohydrate reserves. Although the amount of new roots was positively correlated to the total carbohydrate concentrations averaged over the 2 to 12 week period after outplanting (Figure 2-11), this is not to say that one causes the other.

There was no indication that carbohydrate reserves were used to support root or shoot growth for more than a very brief period after planting. Although TNC decreased during the first two weeks after planting, it increased thereafter. The seedling re-established its normal wintertime pattern of carbohydrate accumulation (Figure 1-9) before substantial growth occurred. As outplanted seedlings caught up and then surpassed nursery

seedlings in total dry weight, they also surpassed them in total reserves. The uncrowded, sunny conditions of field planting apparently became beneficial after an initial phase of reduced dry weight accumulation. Seedlings remaining in the nursery at a density of approximately 30 per square foot were individually exposed to less light and intense competition for soil moisture and nutrients.

The decline in TNC in the first few weeks after planting was relatively small, from 370 mg of non-structural carbohydrate at the time of lifting (based on Chapter I results) to 317 mg after 2 weeks, a decline of only 15%. Moreover, part of the decrease is due to the loss of 30% of seedling root mass and its included reserves during lifting and outplanting. Nevertheless, an important part of the decline must have been due to utilization by the seedling, as is evidenced by a 19% decrease in TNC concentrations (Figure 2-7).

The loss of carbohydrate concentration involved only the starch fraction, specifically root starch. The simultaneous increase in seedling sugar concentrations indicates that starch was being transformed to sugar (Figure 2-5). The reason for the increase in sugar concentrations is unclear.

The transformation of starch to sugar may be related to the internal water balance of the seedling. Water loss or drought is generally regarded as the most serious cause of seedling mortality (Wakeley 1954, Kozlowski 1979). A substantial part of the

absorbing root surface is lost during lifting. Storage, transport, and planting may further desiccate roots. Hence, control of subsequent water loss is essential for seedling survival. During the first 8 weeks after outplanting, the moisture content of seedlings declined (Figure 2-3). The simultaneous increase in sugar concentrations suggested the possibility of free sugars as osmoregulators to check water loss and maintain a positive turgor potential. The role of free sugars as osmoregulators has been discussed by Hsiao et al. (1976) and Turner and Jones (1980).

A capacity for osmotic adjustment would be highly advantageous for outplanted seedlings. By lowering cell osmotic potential, turgor and turgor-dependent processes can be maintained. The result is continued cell enlargement and growth, open stomata, and photosynthesis at water potentials which would otherwise be limiting (Kramer 1983). As noted previously, net photosynthesis presumably began between the second and fourth week after outplanting in a period when the seedlings were still losing water.

The results suggest the sequence of events after outplanting to be: (1) an immediate transformation of starch to sugars used for respiration, metabolism, and possibly osmoregulation; (2) re-initiation of photosynthesis when the internal moisture condition is favorable; (3) re-establishment of the normal seasonal carbohydrate accumulation; (4) expansion of new roots from existing root tips and meristems in various stages of development, both

more abundant in seedlings previously root pruned; and (5) shoot expansion. The critical role of carbohydrate reserves is during the first step when an unfavorable water balance prohibits photosynthesis. By their osmoregulatory ability, carbohydrates can contribute to a favorable internal water potential. The extent of this contribution would depend upon the severity and extent of water deficits.

Nursery undercutting improved seedling performance by affecting one or more of these steps. Upon undercutting, a large portion of the seedling root absorption system is lost. The resulting water deficits would initiate the conversion of starch to sugar for the osmoregulatory process. Repeated undercuttings could result in stable high cell osmotic potentials. These seedlings would be more resistant to water loss when outplanted. A second effect is the increased number of root tips and more fibrous root system of undercut seedlings. This would facilitate water uptake after planting (Rook 1971, Tanaka et al. 1976, Bacon and Bachelard 1978). Nursery undercutting therefore has the potential to increase seedling performance by increasing water absorbing surface area, and by changing seedling physiology so as to decrease water loss due to desiccation.

CHAPTER III THE EFFECT OF WATER STRESS ON SEEDLING MORPHOLOGY AND CARBOHYDRATE PHYSIOLOGY

Introduction

Desiccation after outplanting is the principal cause of initial seedling mortality during plantation regeneration (Wakeley 1954, Kozlowski 1979, Xydias et al. 1981). The definition of a quality seedling necessarily includes the ability to successfully withstand droughty conditions that frequently occur after outplanting. Furthermore, lifting, storage, transport, and planting may also contribute to seedling desiccation.

Seedling morphology is important to drought resistance. For example, low root/shoot ratios are considered to indicate a low ratio of absorbing surface to transpiring surface. But morphological characteristics do not necessarily indicate potential seedling performance after outplanting (Wakeley 1949, Stone 1955, Blair and Cech 1974), and it is generally accepted that seedling quality is a function of physiological condition (Brissette et al. 1981).

Reserve carbohydrate, particularly root starch, has been proposed as an important determinant of physiological quality (Wakeley 1949, Hellmers 1962, Ronco 1973, Barnard et al. 1981).

These reserves would be essential to sustain metabolism such as respiration in the case where post-outplanting moisture stress is sufficient to close stomates and reduce carbon fixation. It is also possible that sugars act as osmoregulators to maintain turgor potential and allow water absorption at lower soil moisture contents (Hsiao et al. 1976, Osonubi and Davies 1978, Kramer 1983).

The present study followed the effect of different levels of water stress on the development of morphological characteristics and carbohydrate reserves of slash pine seedlings in the nursery. The objective was to determine changes in morphological and carbohydrate variables and their possible correlation with seedling quality as determined by survival and growth after outplanting.

Materials And Methods

Nursery

The experiment was executed in the area of the Archer, Florida, nursery previously described in Chapter I (see also Figure 1-1). The experiment required complete control of the amount of water received; therefore, the entire experiment was sheltered by a frame covered with 6 mil (0.006 inch) clear polyethylene plastic. The structure covered three beds, with

only the middle one used for the experiment (Figure 3-1). It was designed so that air movement was not restricted and temperatures at the seedling level were as close to those outside the structure as possible.

The experimental design was a randomized complete block replicated three times (Figure 3-2). Individual plots were 90 cm long separated by a vertical sheet of plastic extending from 2 cm above the bed surface to 30 cm below. This barrier served to restrict the lateral movement of water between plots.

The treatments were defined according to the frequency of watering as well as the total amount received during the 8 week experiment period: December 7, 1982, to January 31, 1983. The five treatments were as follows:

- Treatment 1- watered once at 8 weeks before lifting;
- Treatment 2- watered twice, at 8 and 4 weeks before lifting;
- Treatment 3- watered three times, at 8, 5 and 1 week before lifting;
- Treatment 4- watered four times, at 8, 6, 4, and 2 weeks before lifting;
- Treatment 5- watered each week.

Each application was equivalent to a 2.5 cm rain (28 L/plot). The water was applied using four buckets which slowly dripped out the measured quantity of water over a period of 20 to

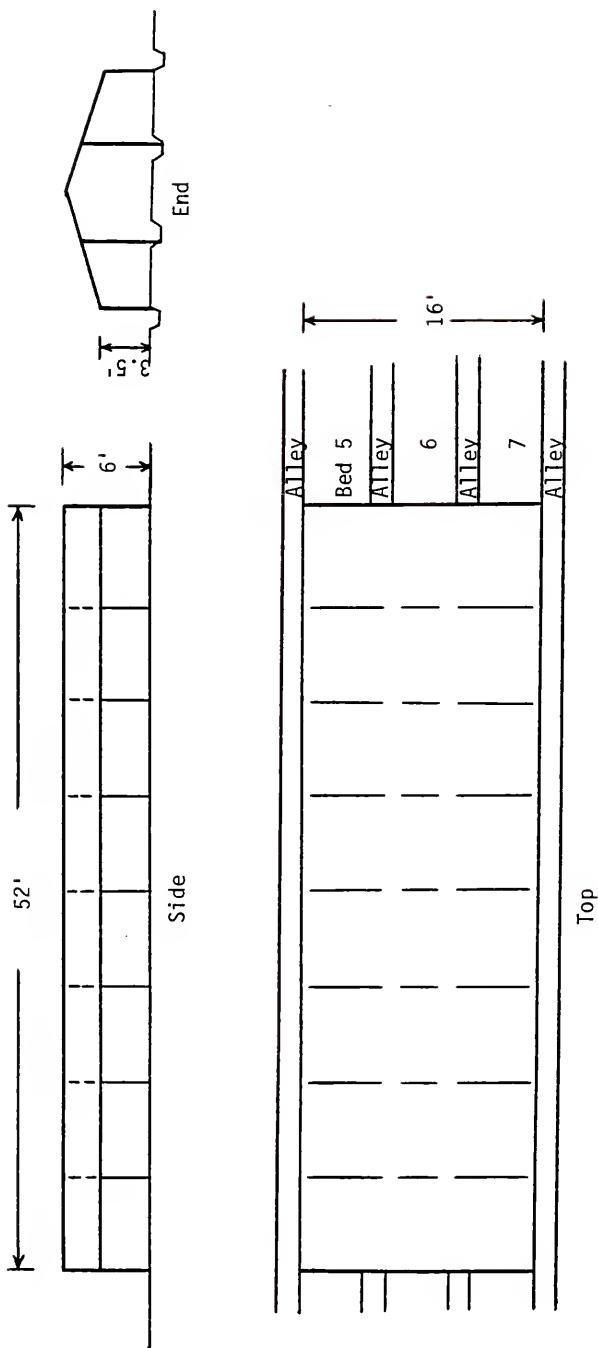


Figure 3-1. Structure covering the water stress experiment at Archer, Florida, nursery. Clear plastic was used for covering material. Sides and ends were open. To scale.

Bed 6, Section 14, Archer nursery

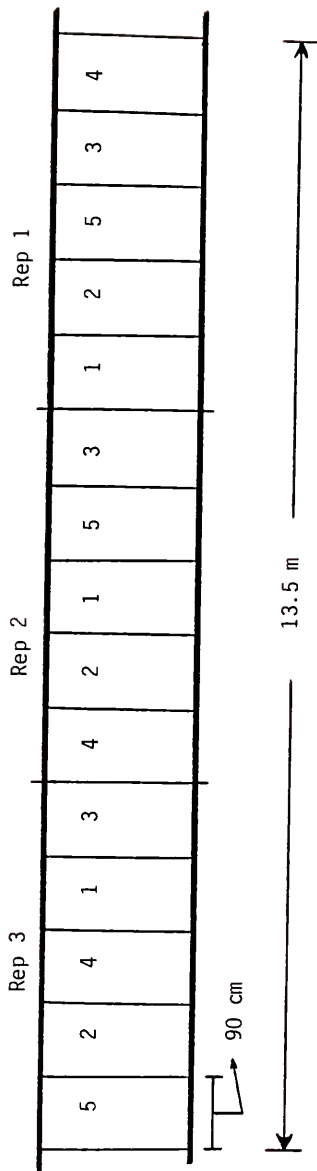


Figure 3-2. Layout of Archer Water Stress Experiment. Number in lower right corner indicates tensiometer depth. Treatments vary by the amount of water received. Each application is equivalent to 25 mm of rainfall.

- Treatment 1 - 8 weeks before lifting;
 2 - 8 and 4 weeks before lifting;
 3 - 8, 5, and 1 week before lifting;
 4 - 8, 6, and 2 weeks before lifting;
 5 - watered weekly.

30 minutes while suspended over a single plot. Because of this long application period and the porous nature of the soil, virtually no water was lost due to surface runoff. The total amounts of water applied were equivalent to 25 mm, 50 mm, 75 mm, 100 mm, and 200 mm rainfall for treatments 1-5, respectively.

A single porous ceramic tensiometer was placed in each plot at 15, 30, or 45 cm depth. Each treatment had a tensiometer at each depth randomly assigned to one of the three replications (Figure 3-3). Tensiometers were installed on December 15 and read weekly thereafter immediately before the scheduled water applications.

Total stem water potentials were determined using the pressure bomb. Whole seedlings were used and all determinations were pre-dawn. A number of measurements were made over the duration of the experiment for different treatments at different times.

At the time of lifting, 2.5 cm diameter soil cores were taken at the specific depth of each tensiometer cup and enclosed in double plastic bags until gravimetric determination of moisture content.

On January 31, twenty seedlings were lifted from rows 3, 4, 5, and 6 in each plot using a shovel. The trees were lifted by cutting laterally through the plot at 17-20 cm depth to simulate the undercutting of normal nursery lifting procedures. Five trees in each row were taken for laboratory analysis (20 total).

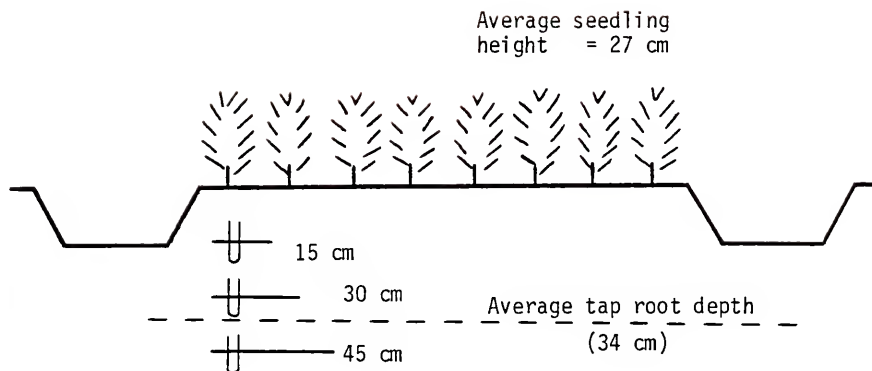


Figure 3-3. Cross section of Bed 6, Section 14, Archer nursery showing average seedling height and tap root depth relative to the three tension-meter depths. To scale.

The remaining 60 seedlings were labeled, bagged with hydromulch, and placed in cold storage at 5°C.

Field

The samples lifted on January 31 were outplanted on February 3. The outplanting location was the same as in Chapter II. Planting was done with a dibble. The soil was moist and weather conditions were cool, clear, and very windy.

The field planting was a randomized complete block with the five treatments planted in rows of 50 seedlings each replicated three times. Nursery replications were continued in the field. A survival count was made at 3 months and height and survival measured 1 year after planting.

Laboratory

Laboratory procedure for the 23 samples taken at the time of lifting was identical to that described in Chapter I with two exceptions. First, the entire 20 tree sample was dried at 105°C for 1 hour and 70°C for 23 hours. Percent moisture was then calculated based on this dry weight. Secondly, all 20 seedlings were used as a composite sample which was subsampled for carbohydrate analysis.

Statistical Analysis

Treatments can be defined either by the total amount of water received during the study or as the number of days since last receiving water. Treatment 1, for example, last received water 55 days prior to lifting. Treatment 2 and 5 both received water 7 days before lifting. When comparing morphological and carbohydrate variability between treatments, a method of analysis was needed to separate the two possible contributions to treatment response. A multiple regression technique of the Statistical Analysis System (SAS) General Linear Models Procedure (Freund and Littell 1981) was used for analysis with the model:

$$\text{Dependent variable} = b_0 + b_1 x_1 + b_2 x_2 + b_3 x_3 + E,$$

Where x_1 = replication,

x_2 = amount of water, and

x_3 = days since receiving water.

This analysis quantifies the contribution of each independent variable to the overall model. The results were also used to determine the direction of further analysis. When amount of water or days since receiving water significantly contributed to the above model, then single factor linear or curvilinear regression was employed to assess how well that factor predicted the dependent variable. This ability was judged by the model R^2 .

Each dependent variable had 15 data points, one from each plot per replication. Morphological values were averages of the 20 tree sample taken at lifting. Carbohydrate values were from analysis of a composite sample of these same 20 seedlings.

Results

Soil Moisture

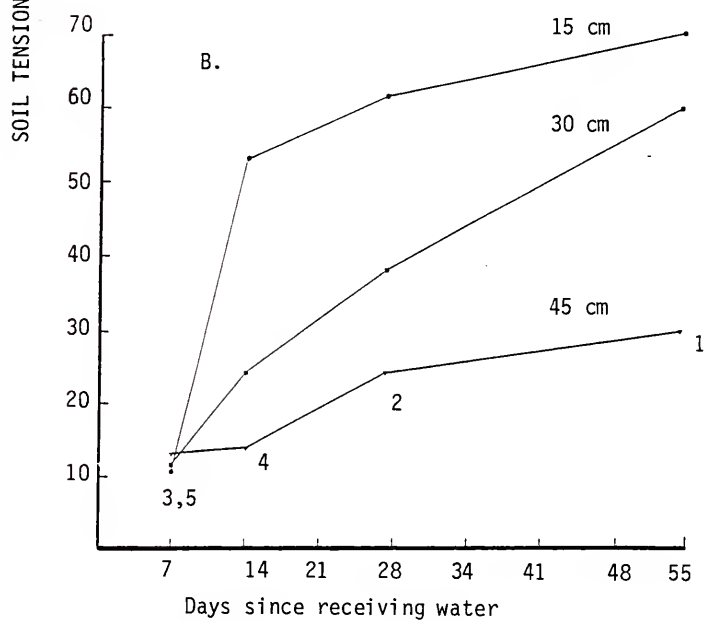
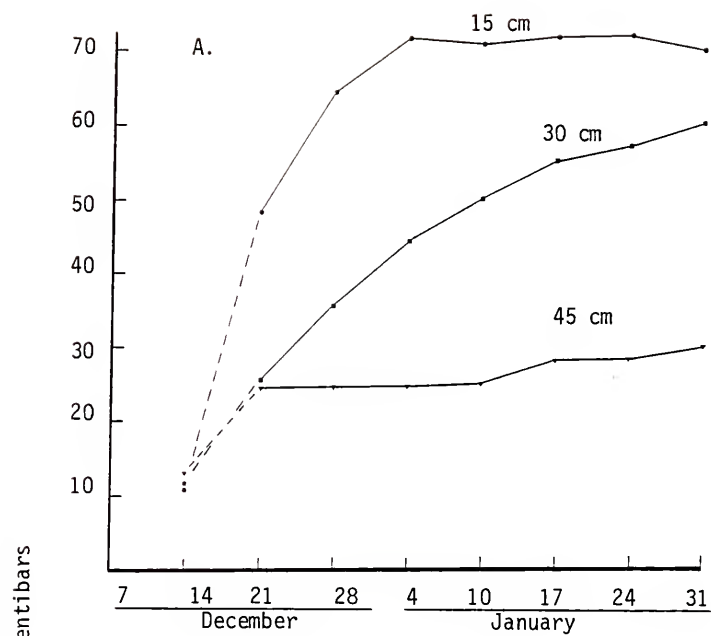
Available soil moisture varied during the 8 weeks of the study as a function of treatment and depth. The similarity between the graphs of Treatment 1 over time (Figure 3-4a) and of all treatments at the time of lifting (Figure 3-4b) reveals that the rate of soil drying after any particular water application was similar for all plots. The decline of available soil moisture was most rapid and severe in the top 15 cm of soil.

The limit of measurability for the tensiometer is around 70 to 80 centibars (McDonald and Running 1979). The 15 cm tensiometer in Treatment 1 reached this point after 28 days. After this point, soil tension measurements at the 15 cm layer are inaccurate. Soil moisture at 15 cm at time of lifting was only 1.2% (dry weight) in Treatment 1, but 6.0% in Treatment 5.

The soil also decreased in moisture content at the 30 cm level, although at a slower rate (Figure 3-4). Even after 55 days without receiving water, the tensiometer reading of

Figure 3-4a. Tensiometer readings at three depths for Treatment 1 on successive dates during the study. Each point represents a single tensiometer reading. Plots received the equivalent of a 25 mm rain on December 7, and were not watered thereafter. Tensiometers were installed on December 15. Values for December 14 were estimated based on other treatments.

Figure 3-4b. Tensiometer readings at three depths, January 31 (lifting date). Number below the line indicate treatments. Each point represents a single tensiometer reading, except for the values at 7 days which is an average from Treatments 5 and 3. The maximum difference between Treatment 3 and 5 at any one depth was 4 cb.



Treatment 1 was 59 centibars (Figure 3-4), corresponding to 1.8% moisture (Figure 3-5).

The loss of soil moisture at 45 cm depth was very slow and most probably inconsequential. This was deeper than the plastic barriers separating treatments and thus some lateral movement of water was possible. When sampled at the time of lifting, Treatment 1 had 3.4% moisture at this depth (Figure 3-5) even though the soil had not been watered for 55 days. Treatment 5, watered weekly, had 4.5% moisture.

Seedling Water Potentials

The number of days since last water application strongly affected seedling stem water potentials (Figure 3-6). Three phases appear to characterize the changes that occurred as soil water became less available. The first was from zero to approximately 10 days when xylem water potential apparently changed very little and remained about -0.2 MPa to -0.3 MPa. From 10 days to 20 days, however, water potentials dropped rapidly, to about -1.1 MPa. At approximately 20-25 days after last receiving water, the rate of decrease slowed. Seedlings which had not been watered for 55 days averaged -1.35 MPa xylem water potential at the time of lifting.

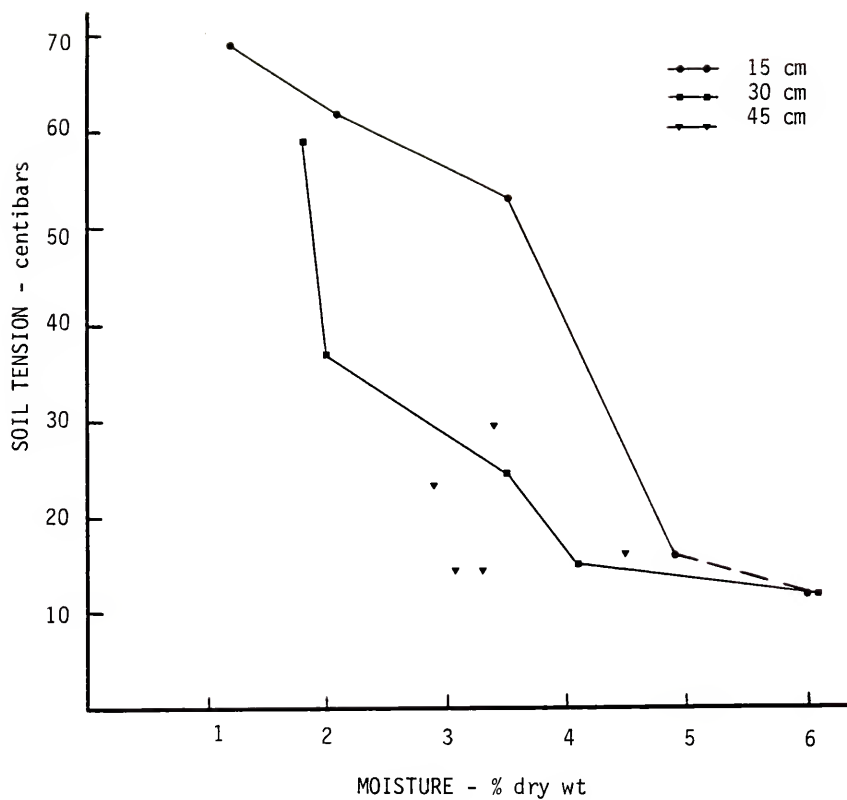


Figure 3-5. The relationship of soil moisture content and soil tension at three depths. Sampled at the time of lifting (January 31).

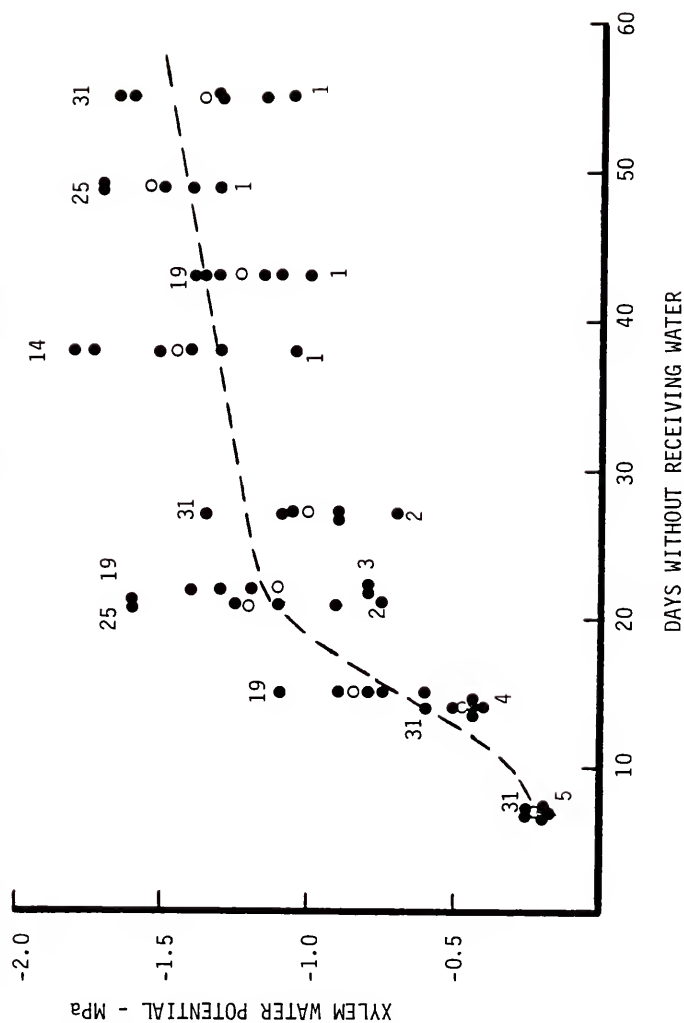


Figure 3-6. The relationship of pre-dawn total stem water potential and the number of days seedlings had not received water. Solid points represent individual seedlings. Circles are means for a specific date. Numbers above points indicate the January date when sampled; Numbers below indicate treatment. Line is hand fitted.

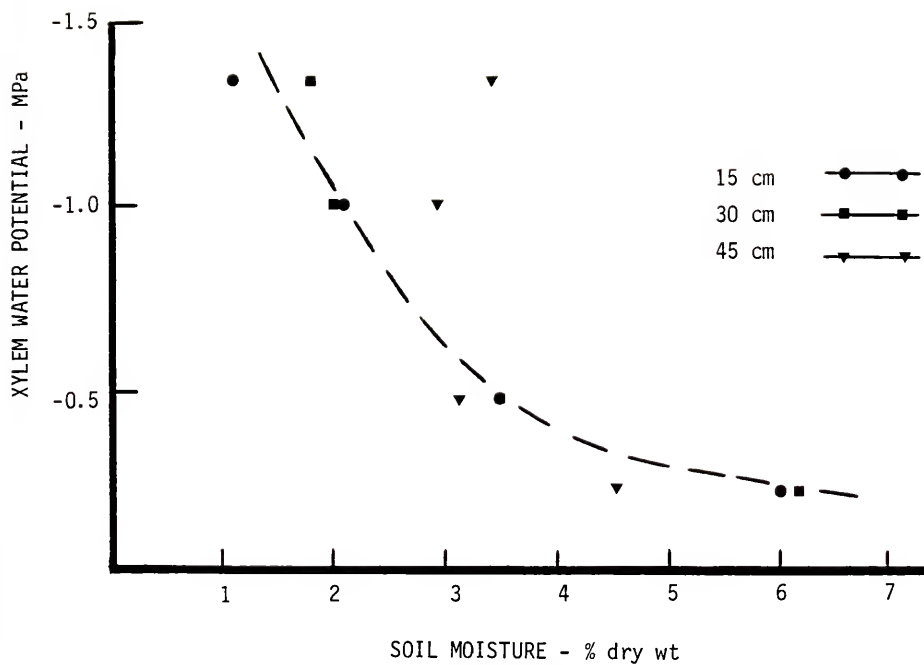


Figure 3-7. The relationship of pre-dawn xylem water potential ($n = 5$) and soil moisture content at time of lifting. Line is hand fitted.

These values are pre-dawn. As significant differences can occur between night and daytime measurements of plant water potentials (Slatyer 1967), xylem water potentials would have become more negative as the atmosphere vapor pressure gradient became more negative during the day.

The total stem water potential varied considerably between seedlings of the same treatment on the same date (Figure 3-6). This may be related to the individual seedling tap root length and the number of absorbing roots in the lower soil horizons where moisture was more available, as well as canopy position and leaf area. Variability among seedlings was less at the higher water potentials. Atmospheric conditions such as dew formation may have influenced results but should have influenced all seedlings equally at a particular sampling time.

Stem water potentials decreased (became more negative) steadily as soil moisture decreased (Figure 3-7). This relationship extended to the 0-15 and 15-30 cm depths, which were similar in content at the time of lifting. Moisture contents at the 45 cm depth were not well related to seedling water potential, but nevertheless were very low.

Seedling Morphological Characteristics

Statistical analysis indicated no significant differences in seedling morphology between treatments at the time of lifting (Table 3-1). Differences between replications, however, were

Table 3-1. Treatment averages for morphological variables measured at the time of lifting.

Variable	Treatment (inches of water)				
	1	2	3	4	8
Height (cm)	28.1	28.6	27.5	27.4	27.4
DRC (mm) <u>1/</u>	3.6	3.4	3.3	3.7	3.7
Fresh weight (g)					
Root	1.09	1.24	1.13	1.64	1.49
Shoot	7.25	6.70	7.09	8.10	6.99
Total	8.35	7.94	8.23	9.74	8.49
Dry weight (g)					
Root	.49	.53	.49	.66	.59
Shoot	2.68	2.62	3.02	2.90	2.71
Total	3.18	3.15	3.52	3.56	3.31
R/S ratio <u>2/</u>	.18	.20	.16	.22	.21
Moisture (% dry wt)					
Root	120	132	126	146	152
Shoot	167	156	137	179	162

1/ Diameter at root collar.

2/ Root dry weight/shoot dry weight.

Table 3-2. Replication averages for morphological variables measured at the time of lifting.

Variable	Replication		
	1	2	3
Height * (cm)	26.4	28.6	28.4
DRC * (mm)	3.2	3.5	3.9
Fresh weight (g)			
Root *	.98	1.29	1.69
Shoot *	5.77	7.58	8.34
Total *	2.66	3.48	3.99
Dry weight (g)			
Root *	.43	.54	.68
Shoot *	2.23	2.83	3.30
Total *	2.66	3.48	3.99
R/S Ratio	.19	.19	.20
Moisture (% dry wt)			
Root	124	138	145
Shoot	158	168	154

* Dependent variable which had a significant contribution of replication effect in the equation $y = a + b_1 (\text{replication}) + b_2 (\text{days}) + b_3 (\text{amount})$ at the .05 (*) or .01 (**) level.

both significant and consistent. Replications decline in the order 3-2-1, for every morphological characteristic except height, root shoot ratio, and shoot water content (Table 3-2). For example, total dry weight declined 25% from Replication 3 to Replication 1.

Carbohydrate Concentrations

Unlike morphological variables, carbohydrate concentrations of root and shoot did not vary by replication. There were, however, consistent and significant effects of treatment (Table 3-3). Both sugar and starch concentrations were functions of water stress. The most intensively stressed seedlings of Treatment 1 had higher sugar concentrations and lower starch concentrations compared to seedlings receiving more water. Total carbohydrate concentrations of Treatment 1 were lower, although the difference is not large.

Normal unmanipulated seedlings from nearby beds increased carbohydrate concentrations, from 109 to 128 mg/g during the 8 week period of this study (Table 3-3). Assuming that experimental seedlings also began at 109 mg/g, all treatments increased total concentrations. Treatment 5 increased by 35 mg/g (32.1%), whereas Treatment 1 increased by only 8 mg/g (8.2%). Overall, sugar and starch concentration in Treatment 3 seemed most similar to levels in adjacent normal nursery seedlings.

Table 3-3. Average carbohydrate concentration at time of lifting.

Variable	Treatment inches of water received					Nursery ^{1/}	
	1	2	3	4	8	Dec 7	Jan 31
----- mg/g dry wt -----							
Sugars							
Root** ^{2/}	93	82	63	69	67	59	55
Shoot**	94	91	81	84	77	81	83
Total**	93	86	71	76	71	70	69
Starch							
Root**	39	73	99	85	123	62	98
Shoot**	9	12	25	15	22	18	19
Total **	24	42	62	50	73	40	59
Total carbohydrate*	117	128	133	126	144	109	128

^{1/} Sample values for unmanipulated seedlings in nearby beds as presented in Chapter I. January 31 values above are an average of January 25 and February 10 results. December 7 values are an average of November 16 and December 15 results.

^{2/} Dependent variable which had a significant contribution of days (since watered) and/or amount (of water) in the equation $y = a + b_1$ (replication) + b_2 (days) + b_3 (amount) at the .05 (*) or .01 (**) level.

Regression analysis indicated that the number of days since water was applied influenced carbohydrate reserves more than did amount of water. For example, Figure 3-8 shows a strong linear relationship between shoot starch concentration and total amount of water received for Treatments 1, 2, 4, and 5. Treatment 3 (75 mm), however, is much closer to Treatment 5 (200 mm). Both received water 1 week prior to sampling. This suggests a restoration of "normal" shoot starch concentrations within 1 week after receiving water.

Certain variables, however, were influenced by total amount of water received during the experiment. Shoot sugars, root starch, total starch and total seedling carbohydrate concentrations all made significant ($\alpha = .05$) model contributions to an equation where the independent variables were replication, days since watered, and amounts of water (Table 3-4).

Even so, the number of days since last receiving water correlated better with nearly all carbohydrate variables (Table 3-4 and Figure 3-9 to 3-11). Only total carbohydrate concentrations were regressed more efficiently (as indicated by R^2 values) using total amount of water as the independent variable. Interestingly, root starch concentrations can be modeled with equal efficiency using either days since watered or amount of water as the independent variable.

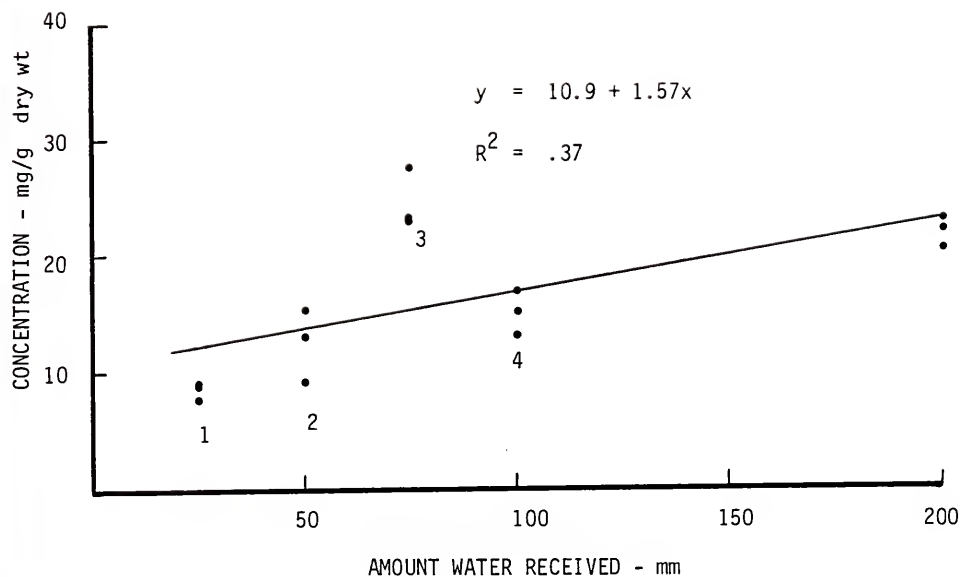


Figure 3-8. The regression of average shoot starch concentration at the time of lifting and the total amount of water received during the 8 week experiment. Each point represents a single replication/treatment combination (20 seedling sample). Numbers below the points indicate treatment.

Table 3-4. Best fitting models for carbohydrate concentrations at time of lifting ^{1/}. (Days = days since last receiving water; Amount = total amount of water received.)

	<u>R²</u>
Root sugar conc. = $57.5 + 1.02(\text{days}) - .007(\text{days})^2$.86
Shoot sugar conc. = $72.57 + .96(\text{days}) - .01(\text{days})^2$.86
Average sugar conc. = $65.04 + .99(\text{days}) - .008(\text{days})^2$.92
Root starch conc. = $115.63 - 1.44(\text{days})$.83
Root starch conc. = $21.0 + 26.5(\text{amount}) - 1.75(\text{amount})^2$.83
Shoot starch conc. = $28.37 - .90(\text{days}) + .01(\text{days})^2$.85
Average starch conc. = $69.31 - .86(\text{days})$.85
Total carbohydrate conc. = $118.48 + 3.22(\text{amount})$.55

^{1/} All models are statistically significant for the test $B \neq 0$ at the .01 level (Freund and Littell 1981).

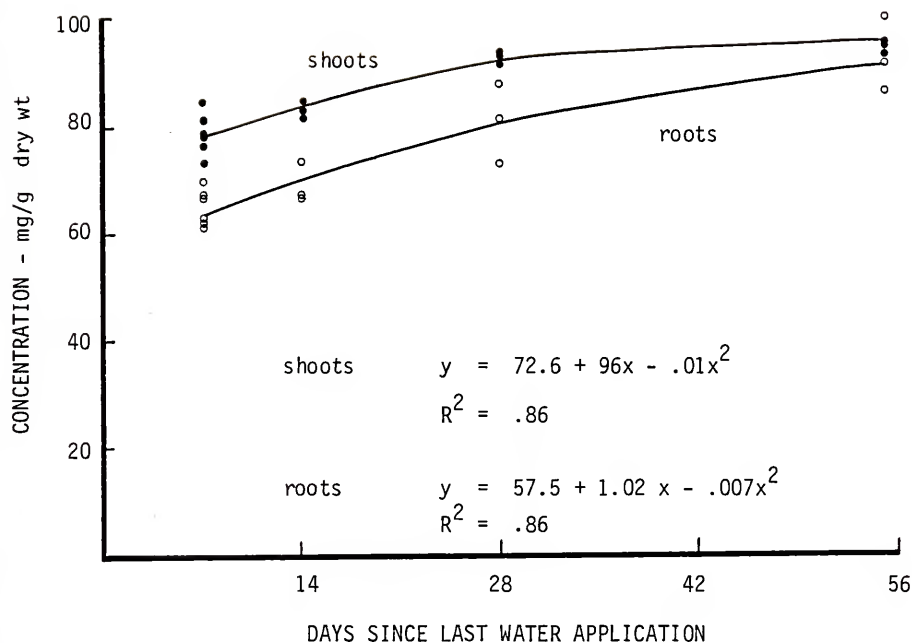


Figure 3-9. The regression of seedling root and shoot sugar concentrations and the number of days without receiving water prior to sampling. Each point represents a single replication/treatment combination (20 seedling sample).

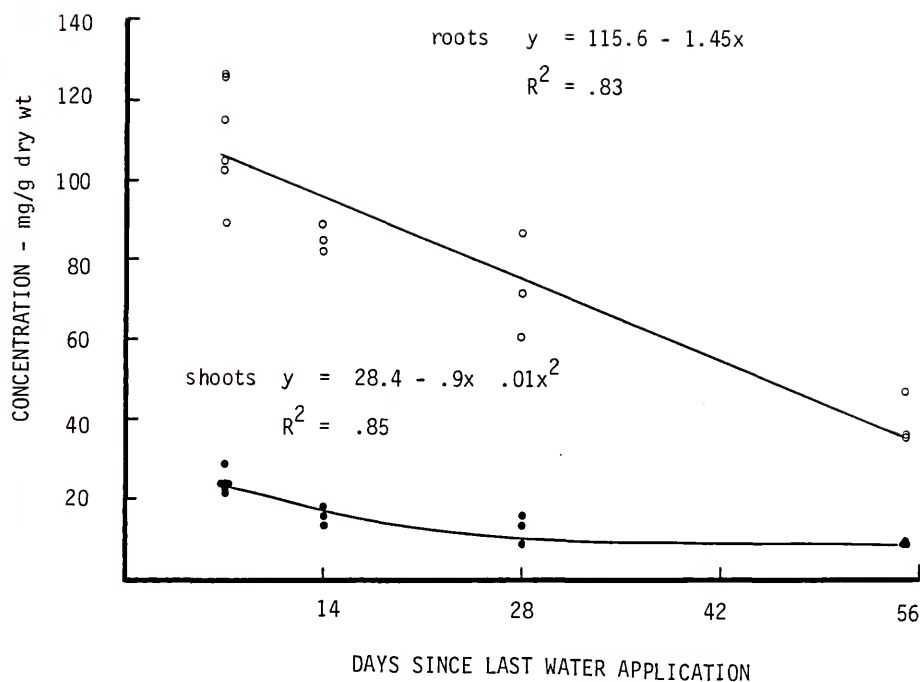


Figure 3-10. The regression of root and shoot starch concentrations and the number of days without receiving water prior to sampling. Each point represents a single replication/treatment combination (20 seedling sample).

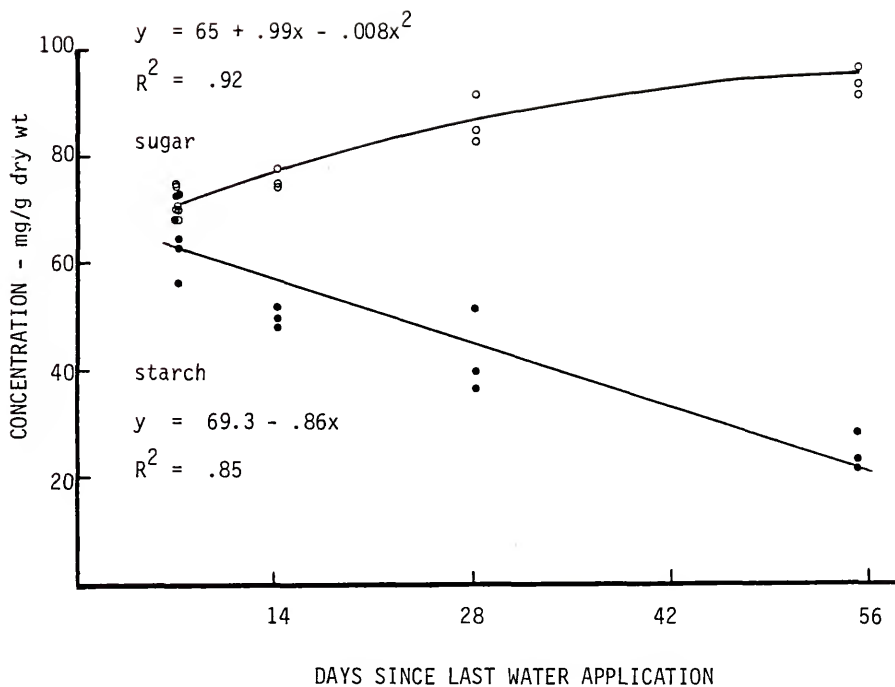


Figure 3-11. The regression of whole seedling sugar and starch concentrations and the number of days without receiving water before lifting. Each point represents a single replication/treatment combination (20 seedling sample).

In general, total carbohydrate concentration decreased with increasing water stress, but its variability was not adequately accounted for by any model. While the statistical procedure indicated high significance for the test of $B \neq 0$, the model R^2 of .55 indicates much unexplained variability.

Amount of Carbohydrate per Seedling

Multiple regression analysis of the dependent variables, i.e., total sugars, total starch, and total carbohydrate, against the independent variables, i.e., replication, days since watered, and amount of water, revealed that replications contributed significantly to the models of all three dependent variables. This effect is a consequence of seedling size, which varied significantly between replications (Table 3-2).

The amount of sugar per seedling increased with increasing water stress from 251 mg/seedling for Treatment 5 to 298 mg/seedling for Treatment 1, an increase of 19% (Table 3-5). The amount of starch decreased, from 134 to 43 mg/seedling, a 70% reduction in absolute amounts. total carbohydrate per seedling decreased from 385 to 341 mg/seedling, or 11%. The statistical analysis was able to separate treatment effects only for the relatively large changes in starch.

Treatments 1 and 2 are distinguished from 3, 4, and 5 by lower total carbohydrate, with a lower proportion as starch and a higher proportion as sugar (Table 3-5). The carbohydrate levels

Table 3-5. Average mass of sugar, starch, and total carbohydrate per seedling at lifting.

	Treatment (inches of water received)					Nursery ^{1/}	
	1	2	3	4	8	Dec 7	Jan 31
Sugar (mg)	298	283	277	289	251	232	272
Starch	43	71	122	99	134	83	142
Total	341	354	399	388	385	315	414
Sugars as a % of total	87	80	70	74	65	74	66
Starch as a % of total	13	20	30	26	35	26	34

^{1/} Sample values for unmanipulated seedlings in nearby beds as presented in Chapter I. January 31 values above are an average of January 25 and February 10 results. December 7 values are an average of November 16 and December 15 results.

of Treatments 3 and 5 are comparable to those in nearby unmanipulated seedlings that were not under the experimental rain shield. These seedlings had both similar total amounts of non-structural carbohydrate and similar partitioning of carbohydrates into sugars and starches. These similarities indicate that the rain shield did not appreciably affect carbohydrate accumulation.

Field Performance after Outplanting

Overall survival averaged 92% (Table 3-6). Low mortality was likely a result of adequate rainfall inasmuch as the site received a minimum of 12 mm rainfall every week from January 20 to March 31 (planting was on February 3), with an average of 35 mm per week.

Due to high survival, there was no significant effect of either replication or treatment. A linear model which tested survival as a dependent variable against the three independent variables of replication, days since watered, and amount of water revealed no significant influence of any variable.

Using the same model, however, height at 1 year after planting varied significantly by both replication and days since last watered. Average heights for replications 1, 2, and 3 were 30.0, 32.6, and 33.6, respectively. Yet, when height increment was used as the dependent variable, replication had no effect.

Table 3-6. Treatment averages for survival, height, and height increment 1 year after planting.

	Treatment (inches of water received)				
	1	2	3	4	8
Survival (%)	91	93	94	95	89
Height (cm)	29.0	33.0	33.1	32.1	32.8
Height increment (cm)	.9	4.4	5.6	4.7	5.4

Hence, although taller nursery seedlings were still taller after 1 year in the field, height growth during that year was not related to their initial height.

One year height also varied by treatment, although only the most severely stressed seedlings of Treatment 1 were markedly shorter (Table 3-6). The regression of field height on number of consecutive days without receiving water prior to lifting in the nursery, was significant at the .05 level. An R^2 of .32, however, indicates a large amount of unaccounted-for variability.

Treatment effect was more evident when measured as first year height increment, with an increase from 0.9 cm for Treatment 1 to 5.4 cm for Treatment 5 (Table 3-6). The relationship of increment to number of days since last watered prior to outplanting was approximately linear and significant at .01 for $b \neq 0$ (Figure 3-12), with an R^2 of 0.57.

An attempt was made to account for treatment effects on increment by regression against morphological and carbohydrate data taken at the time of lifting (Table 3-7). The carbohydrate variables were better predictors of first year height increment than were the morphological variables.

The strongest linear variables were root starch content and total seedling starch content (Figure 3-13, Table 3-7). Since both are products of dry weight x concentration, they combine a morphological characteristic with concentration. Dry weight alone, however, is only weakly correlated with first year height increment (Table 3-7).

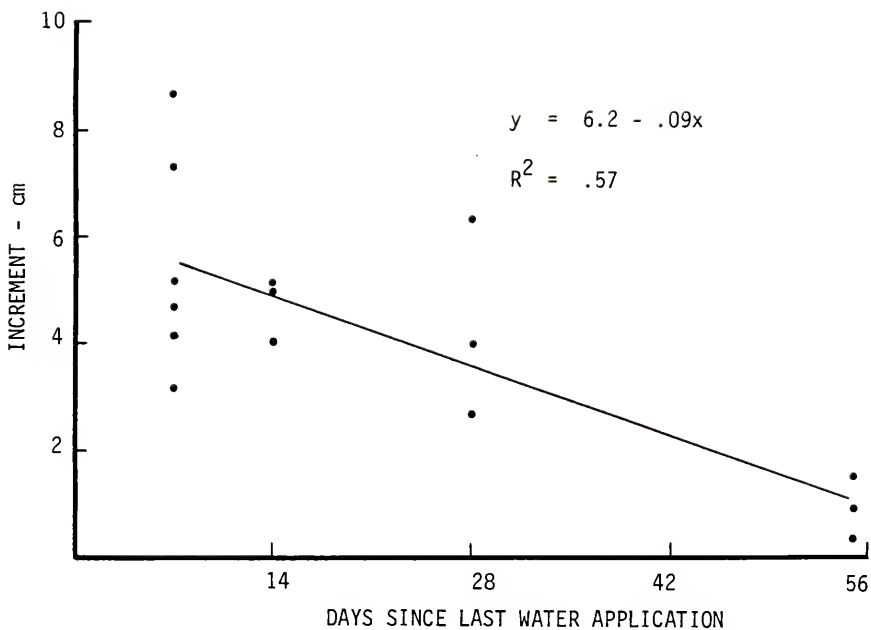


Figure 3-12. The regression of first year height increment and the number of days seedlings had not received water prior to lifting. Each point represents a single replication/treatment combination (50 seedling sample).

Table 3-7. Independent variables tested in linear regression against the dependent variable of the first year height increment.

Independent variable	probability of $B \neq 0$	R^2
Total starch mass	.01	.67
Total root starch mass	.01	.66
Root starch concentration	.01	.59
Total starch concentration	.01	.56
Total root carbohydrate mass	.01	.48
Total carbohydrate concentration	.01	.46
Total carbohydrate mass	.02	.35
Total root carbohydrate/shoot dry weight	.02	.35
Root sugar concentration	.02	.34
Total sugar concentration	.02	.33
Shoot starch concentration	.03	.32
Root dry weight	.05	.26
Total dry weight	.05	.26
Shoot dry weight	.06	--
Shoot sugar concentration	.07	--
Total shoot carbohydrate	.07	--
Height/DRC	.15	--
DRC	.26	--
R/S ratio	.32	--
Height at lifting	.69	--

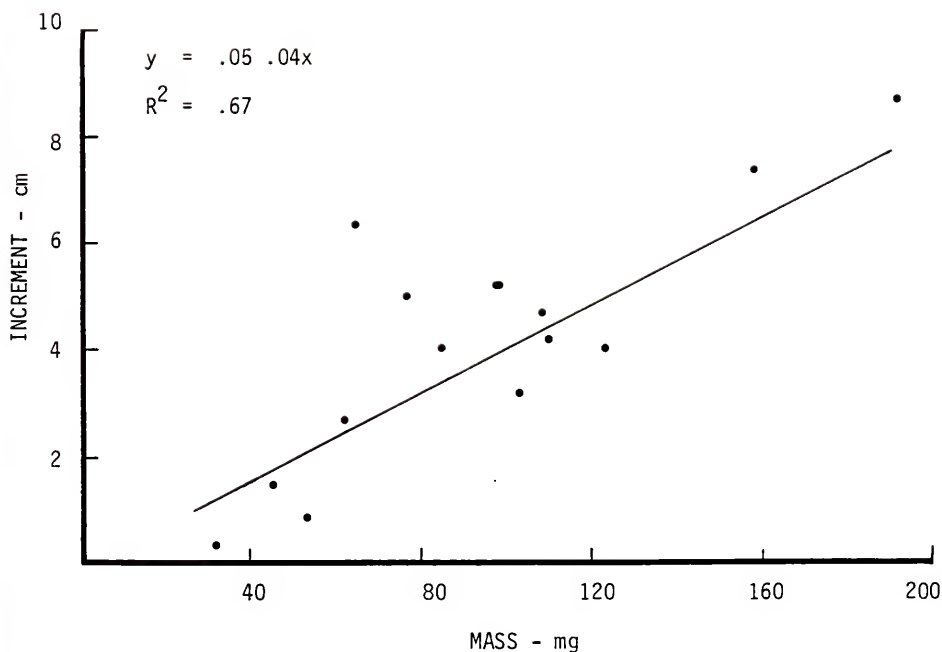


Figure 3-13. The regression of first year height increment and the absolute amount of starch per seedling at the time of lifting. Each point represents a single replication/treatment combination.

The best independent indicator of seedling performance was root starch concentration. It was associated with 59% of the variability in first year increment. Inclusion of root dry weight in the model increased predictability only slightly (Table 3-7).

Discussion

Seedling Growth

Pre-dawn xylem water potential on January 31 varied from -0.2 MPa to -1.6 MPa (Figure 3-6). Basic seedling physiological processes must have been affected at the lower water potentials. Water stress is known to cause a number of physiological effects, including an increase in abscisic acid concentrations and decreases in protein synthesis, protochlorophyll formation, and cell wall synthesis (Hsiao et al. 1976). The photosynthetic rate of loblolly pine has been shown to rapidly decline at leaf water potentials below -0.4 MPa, ceasing completely at -1.1 MPa (Brix 1962). The first effect of even minimal water stress is a decrease in internal turgor pressure and a slowing or cessation of growth (Hsiao et al 1976, Kramer 1983). Physiological response, however, is dependent upon pre-stress conditioning (Osonubi and Davies 1979, Seiler 1984).

In this study, lower xylem water potential was a direct result of decreasing soil moisture availability, especially in the upper top 15 cm of soil where seedling roots were concentrated (Figure 3-7). At other periods of the year the rate of soil moisture loss would be higher because of greater evapotranspiration (ET). Moreover, the slight reduction in radiation by the rain shield would have slightly reduced potential ET.

No consistent morphological differences among treatments were measured at time of lifting (Table 3-1). This is certainly related, in part, to the growth stage when treatments were imposed, and the relatively slow growth rate during the 8 week experiment. For example, seedling root growth of unmanipulated seedlings is only 15 mg dry weight per week during this season (Table 1-1). Given the amount of experimental error, a longer time period would be necessary to produce measureable effects under these experimental conditions.

The variation in seedling size among replications was most likely related to differences in soil drainage. The smaller seedlings were found towards the end of the bed where water tended to accumulate after heavy rains. No such rains occurred during this experiment, however, and the size variation was the result of pre-existing conditions.

Seedling Carbohydrates

Seedlings increased sugar concentrations at the expense of starch concentrations when subjected to drought. This phenomenon has been observed in seedlings of sugar maple (Acer saccharum March.)(Parker 1970) and black oak (Quercus velutina Lam.)(Parker and Patton 1975). In both cases, root starch decreased with increasing water stress. In black oak, there were simultaneous increases in concentrations of sugar and several amino acids.

In the present study, the differences in carbohydrate concentrations resulting from varying levels of moisture stress were consistent among seedlings of different sizes. Replications varied in seedling size but not in carbohydrate concentration, indicating that seedlings responded to moisture stress in the same manner, regardless of size.

Stomatal closure may be the immediate result of even small amounts of water stress (Brix 1962, Lopushinsky and Klock 1974). In this case, maintenance of metabolic activity depends upon storage carbohydrate. This could explain the decrease in starch concentrations observed under the stress conditions of this study. It does not, however, explain the simultaneous increase in sugar concentration (Figure 3-11).

Sugars can be used for osmotic adjustment to maintain internal water potentials (Kramer 1983). Osmotic adjustment appears to be an important mechanism for adaptation of some mesophytic plants to water-limiting conditions (Hsiao et al. 1976).

By increasing solute concentrations in cells, turgor potential--and therefore cell expansion--can be maintained.

Reports of osmotic regulation in agronomic crops are fairly common (Eaton and Eargle 1948, Greacen and Oh 1972, Meyer and Boyer 1981). For example, the growth rate of 3-5 day old pea seedlings (Pisum sativa L.) was constant through a range of soil moisture tensions from -0.28 MPa to -0.83 MPa. This was attributable to the maintenance of a stable pressure potential through the reduction of solute potential by as much as -0.7 MPa (Greacen and Oh 1972).

The maintenance of turgor pressure by osmotic regulation has been demonstrated also in woody plants. Osonubi and Davies (1978) found that increasing solute concentrations in roots and shoots of English oak (Quercus robur L.) seedlings were associated with decreasing soil moisture. This osmotic adjustment resulted in "maintenance of turgor and high leaf conductance as the soil dried." Loblolly pine was shown to produce a -0.4 MPa osmotic adjustment when subjected to moderate water stress (total stem water potential of -0.75 MPa) (Hennessey and Daugherty 1984).

The results of the present study suggest that slash pine seedlings undergo osmotic adjustment by increasing sugar concentrations. Starch reserves were transformed to sugar, accounting for the simultaneous increase in sugar concentration and

decrease in starch concentration while overall carbohydrate reserves decreased only slightly. Although starch mass decreased by 70% from Treatment 5 to Treatment 1, total carbohydrate decreased by only 18% (Table 3-6).

Amino acids (Parker and Patton 1975) and specific mineral ions (Ford and Wilson 1981) may also be used for osmotic adjustment. In both cases, respiratory substrate (carbohydrate) is necessary. Respiration supplies the metabolic precursors for amino acid synthesis and the energy for active transport of mineral ions. Therefore, any increase in sugar concentration may be a direct result of water stress, but an indirect contributor to osmotic adjustment.

The continuing reduction in xylem water potential slowed at about 30 to 40 days after last receiving water (Figure 3-6). At this point, sugar concentration also began to level off (Figure 3-9) and the limit of any sugar contribution to osmotic adjustment may have been reached. Because xylem potential leveled off, it is likely that the stomates remained closed at this time. Therefore, seedlings depended solely upon such morphological adaptations as a heavy cuticle to slow moisture loss.

Although sugar concentration began to stabilize at around 30 days, starch concentrations continued to decrease (Figure 3-11). This may be related to respiratory demands. In the case of loblolly pine, respiration first decreased then dramatically increased at about -1.6 MPa leaf water potential (Brix 1962).

Assuming that seedlings began with 109 mg/g total carbohydrate on December 7, total carbohydrate concentrations (Table 3-3) and mass (Table 3-6) increased over the period of the experiment. These increases were small in the most severely stressed seedlings of Treatment 1. Nevertheless, any increase indicates that the photosynthetic process was active during at least part of this 8 week period, most likely during the first few weeks in the case of Treatment 1. The contribution of osmotic adjustment to continued photosynthesis during the subsequent weeks is unknown.

It is possible that the lack of statistical differences in morphological characteristics among treatments is related to osmotic adjustment. In principle, lower seedling osmotic potential would allow for continued water uptake at lower soil moisture tensions.. The amount of water made available by such osmotic adjustment, however, would depend not only upon the ability to increase cell solute concentration but on the amount of water potentially available in the soil. The relatively small content of non-gravitational water and low hydraulic conductivity of these soils limits the effectiveness of osmotic adjustment as a mechanism to influence water uptake. Thus, osmotic adjustment serves mainly to slow seedling desiccation. In clay or loamy soils, osmotic adjustment might have more influence on seedling water absorption.

The starch to sugar interconversion is very responsive to rewatering. This is indicated by the strong relation between days since watered and sugar and starch concentrations. For example, Treatments 3 and 5, which had both received water 1 week prior to sampling, had similar values for sugar and starch concentrations. Yet Treatment 3 had received a total of 75 mm of water vs. 200 mm for Treatment 5 during the 8 week experiment.

Rapid responses of carbohydrate physiology to environmental stimuli is not unusual. Sands et al. (1984) reported that potted radiata pine seedlings, dried to -1.6 MPa xylem water potential, returned to 100% of their pre-stress photosynthetic levels within 3 days after rewatering. The ability to respond to rewatering varied among families.

The apparent sensitivity of starch and sugar concentrations to moisture stress should be considered in any sampling procedure where carbohydrates are of interest. Studies using starch or sugar concentrations as a parameter in seedling evaluation need to consider their rapid interconversion as a function of seedling water potential.

Outplanting

Although no statistically significant effects of treatments upon morphological parameters were measured at the time of lifting, treatments nevertheless had long-term effects on seedling development. The site of this experiment was revisited 5 weeks after completion of the study and removal of the rain shield.

Length of the spring shoot, although not measured, varied in accord with the previous treatments. Seedlings subjected to higher moisture stress had shorter shoots than those seedlings in the surrounding nursery beds. Hence, the water stress in December and January caused a reduction in spring stem elongation nearly 2 months later.

The effects of treatments on height increment of outplanted seedlings presumably reflected the same phenomenon. During December and January, 65 to 75% of the nursery seedlings lacked winter buds (Chapter I), indicating a quiescent state of bud development. Water stress applied at this time probably affected bud development by reducing the number of anatomical stem units (Lanner 1971) leading to smaller than normal shoots after expansion.

Lowered carbohydrate levels also may have reduced initial height growth. If the elongation of the spring shoot is dependent upon starch reserves, then a reduction in reserves would decrease its length. The depletion of stored reserves during shoot elongation of conifers has been shown for red pine (Olofinboba and Kozlowski 1973) and Douglas-fir (Kreuger and Trappe 1967).

Carbohydrate variables, and in particular, root starch concentrations showed the strongest correlation with first year height increment. Survival, on the other hand, was not related

to root starch levels, even though concentrations varied from 39 to 123 mg/g dry weight. This most likely reflects the excellent weather after planting.

Whatever the mechanisms, severe moisture stress imposed in the nursery reduced height growth during the first growing season in the field even though planting conditions and rainfall were favorable. Bud development and carbohydrate reserve levels could have had confounded effects upon spring shoot elongation. Because reserve levels were quantified, their contribution to seedling performance was determined and root starch concentration was found to be a good predictor of seedling performance based on first year height increment.

The planting location of this experiment was a well- to excessively-drained, sandy soil which had been bedded. As such, moisture availability may have been limiting for slash pine seedlings on this site during the first year. By planting on a difficult site, variability in physiological quality may have been made more apparent. Better site conditions might have reduced or eliminated height increment differences among treatments if the seedlings had been able to increase the frequency or size of recurrent flushes during the summer.

One can only speculate as to the possible influence of the nursery treatments if planting conditions had been less favorable. Seedling ability to respond to rewatering is dependent upon

the stress duration (Brix 1962, Zavitkovski and Ferrell 1970, Kramer and Kozlowski 1979). If the seedlings had been subjected to higher levels or longer periods of water stress after out-planting, survival could have been affected. If sugars are used for osmotic adjustment, the ability to recover from stress would have varied among treatments according to their reserve levels.

SUMMARY AND IMPLICATIONS

Summary of Findings

The overall objective of this study was to investigate the relationship between carbohydrate reserves and the quality of slash pine seedlings grown in northern Florida nurseries. The investigation revealed several ways in which carbohydrate reserves relate to seedling quality.

Experiment 1 revealed the seasonal pattern of reserve carbohydrate accumulation in seedlings growing under favorable conditions. There were large increases of reserves, especially root starch from November to February (Figures 1-8 to 1-10). Average non-structural carbohydrate concentration increased from 79 mg/g dry weight on October 19 to 145 mg/g on February 24 (Figure 1-9). During this period, root starch concentrations increased from 8 to 128 mg/g (Figure 1-8).

Seedling mass also increased greatly in late fall and winter, though height did not. Root dry weight increased from 495 mg on October 19 to 1218 mg on February 24. Shoot dry weight

increased from 2083 to 2731 mg in this period. Hence, physiological activity continued throughout the normal nursery lifting season with an increasing proportion of current photosynthate allocated to root expansion and carbohydrate reserves.

The second experiment showed that seedlings can rapidly re-establish nursery growth patterns after lifting and planting. Carbohydrate accumulation began again 2-4 weeks after planting in January regardless of undercutting treatment, and in the same pattern as seedlings remaining in the nursery (Figure 2-7). Analysis revealed a linear increase in seedling weight from 0 to 12 weeks after planting (Table 2-3). The extent of post-planting development was undoubtedly influenced by environmental factors, i.e., favorable rainfall.

Undercutting in the nursery had profound effects on seedling root morphology. Intensively undercut seedlings had six times more new root mass at lifting than the controls (Table 2-2). Treatment effects continued after outplanting, with the intensively wrenched seedlings producing significantly more new roots than the controls during the first 12 weeks (Figure 2-4, Table 2-4).

The production of new roots affected first year survival and height growth. New root growth in the first 12 weeks after outplanting correlated with first year survival ($R^2 = 0.48$).

First year survival and height increment also increased significantly with intensity of undercutting in the nursery (Figure 2-8 and 2-9).

The relationship of carbohydrate reserves to superior seedling performance in experiment 2 is difficult to assess. Frequently undercut seedlings contained more reserves than the controls during the first 12 weeks after planting and new root growth was correlated with carbohydrate mass, $R^2 = 0.50$ (Figure 2-11). It is doubtful that root expansion after planting depended on accumulated reserves, however, because both mass and concentration of carbohydrates increased at the same rate in outplanted seedlings as in those remaining in the nursery (Figure 2-2). Active photosynthesis must have supported root growth and reserve storage during the first 12 weeks after planting.

A possible influence of reserves to seedling development after outplanting occurred in the first two weeks. During this time, root starch concentrations fell by about 40% (Figure 2-6). The fate of these reserves is uncertain. Yet, simultaneous to the decrease in seedling starch concentration was an increase in seedling sugar concentration (Figure 2-5) relative to seedlings remaining in the nursery.

The apparent transformation of starch to sugar occurring after outplanting was also observed when seedlings were water stressed in the nursery. A simultaneous decrease in starch and

increase in sugar concentration was correlated with increased water stress (Figure 3-11). The results suggest that seedlings used free sugars to osmotically adjust to decreasing soil moisture availability. Increased respiration rates (Brix 1962) and amino acid synthesis (Parker and Patton 1975) have also been associated with plant water deficits and could have been a stress induced drain on increased sugar concentrations.

Severe moisture stress in the nursery reduced height increment in the first year after outplanting (Figure 3-12, Table 3-6). This was most probably due to limited bud development during December and January when stress occurred. It is possible that a stress-caused decrease in starch concentration also contributed to reduced growth, however, as there was a strong correlation between first year height increment and total seedling starch at the time of lifting (Figure 3-13).

Practical Implications

Definition of seedling quality should be expanded to consider carbohydrate status during the lifting season and any post-lifting storage. In the past, morphological characteristics have been the prime determinants of quality (Wakeley 1954, Brissette et al. 1981), and it is generally accepted that there are "normal" morphological attributes indicative of "plantability."

The present study shows that "normal" slash pine seedlings have a definite pattern of carbohydrate accumulation from October to February. Deviation from this norm indicates something is awry, and thus survivability may be compromised.

Seedlings are not dormant during the late fall and winter months. This is demonstrated by the continued weight increases and carbohydrate accumulation (Chapter I), root growth after outplanting (Chapter II), and starch-sugar interconversion (Chapter III). Seedlings actively respond to undercutting in November and December or to severe water stress in December and January. Presumably, they might also respond to other cultural treatments such as fertilization.

Reserves are relatively low during October, November, and December (Figure 1-10). Therefore, the period between lifting and planting should be minimized so as to reduce respiratory loss of reserves (Hellmers 1962, Ronco 1973, McCracken 1979). Seedlings are more storable in January and February when their total non-structural carbohydrate reserves are highest.

October through December are normally the driest months of the year in northern Florida (Dohrewend 1978). Early lifting therefore combines normally low rainfall with low seedling starch reserves. Low starch concentration limits seedling capacity for increasing sugar concentrations (Chapter III). The possible result is dry field conditions and a seedling with a reduced

capacity to adjust to desiccation. Coordination of lifting, storage, transport, and planting is most critical during this period in order to maximize reserves and minimize desiccation.

Fall undercutting might improve the performance of early lifted seedlings by preconditioning them to withstand dry field conditions. Undercutting is a regularly used method of improving seedling performance in other conifers (Van Dorsser and Rook 1972, Bacon and Hawkins 1979). It has been shown to physiologically condition seedlings for outplanting (Bacon and Bachelard 1978). Morphological changes caused by undercutting are also related to improved seedling performance (Rook 1971).

In the present study, undercutting significantly improved seedling performance. Although the results are limited to 1 year of data and undercutting only in November and December, the differences indicated substantial improvement in seedling performance. Many nurserymen hesitate to use undercutting as a standard procedure for fear of root pathogens. If this fear seems warranted, possibly only those parts of the nursery destined for early lifting would be undercut. In any event, undercutting should be looked at more thoroughly as a cultural technique to improve seedling quality.

Stressing seedlings in the nursery by withholding irrigation is widely used to "harden-off" seedlings before outplanting.

This study has shown, however, that an extended period of stress can be detrimental to seedling performance. Based on the results of Chapter III, and others (Bacon and Bachelard 1978, Seiler 1984), the objective of the nurseryman might be better served by conditioning seedlings through cycles of stressing and watering.

Seedlings were able to adjust to about -1.0 MPa xylem water potential (Figure 3-6), corresponding to about 60 centibars soil water tension. Beyond this point, stomates closed and productivity must therefore have declined. By monitoring soil water potential, the nurseryman could water when the soil dried to this tension. This technique of seedling conditioning is obviously limited by amount and frequency of rainfall.

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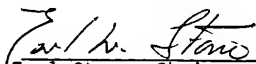
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BIOGRAPHICAL SKETCH

Kenneth Lee McNabb was born November 20, 1949, in Robinson, Illinois, the son of Anita Mae and Kenneth Jackson McNabb. He attended Lincoln Grade School and Robinson High School, graduating there in 1967. Upon entering Southern Illinois University the same year, he began professional training in forestry and received the Bachelor of Science degree in the area of forest recreation in 1971 and the Master of Science in the area of silviculture in 1973. Data for the masters thesis were taken while on a 1 year stay in southern Brazil. From 1974 to 1980 he was employed by Jari Forest Products, an American-owned company producing paper from tree plantations in the Amazon basin. He left Jari in order to pursue further graduate studies at the University of Florida, and received the Ph.D. Degree in the area of tree physiology in 1985.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.



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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.




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